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THE JOURNAL

OF

EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D. PEYTON ROUS, M.D.

VOLUME XXXVIII, No. 4 OCTOBER 1, 1923

PUBLISHED MONTHLY

AT MOUNT ROYAL AND GUILFORD AVES., BALTIMORE, MD.

BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

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Entered as second-class matter June 3, 1916, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Accepted for mailing at special rate of postage provided for in section 1103, act of October 3, 1917.
Authorized June 29, 1918.

Made in the United States of America

PUBLICATIONS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

THE JOURNAL OF EXPERIMENTAL MEDICINE

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WAVERLY PRESS
WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

BOTULISM.

STUDIES ON THE MANNER IN WHICH THE TOXIN OF CLOSTRIDIUM BOTULINUM ACTS UPON THE BODY.

II. The Effect upon the Voluntary Nervous System.*

By ERNEST C. DICKSON, M.D., and ESHREF SHEVKY, Ph.D.

(From the Laboratory of Experimental Medicine of Stanford University Medical School, San Francisco.)

Plate 24.

(Received for publication, June 4, 1923.)

In a previous report (1) it was shown that the *botulinus* toxin produces a peculiar blocking of nerve impulses in the parasympathetic fibers which have been described by Gaskell (2) as the prosomatic and bulbosacral outflows of connector fibers of the involuntary nervous system, but that the true sympathetic fibers, the thoracicolumbar outflow, are not affected. As will be shown later in this report this blocking of nerve impulses adequately explains many of the clinical manifestations of botulism but there are certain other symptoms and signs which cannot be so explained.

Preliminary experiments by one of us (3) had shown that there is no apparent damage to the cell protoplasm when various bacteria, strips of frog pharynges, frog gastrocnemius muscles, or guinea pig muscles are immersed into the filtrate of a broth culture of *Clostridium botulinum* which contains a virulent toxin, and that if corresponding excised thigh muscles of normal guinea pigs and guinea pigs which have been poisoned with *botulinus* toxin are stimulated directly by repeated tetanizing stimulations, no difference can be demonstrated in the sen-

* These experiments are a part of an investigation of botulism which was made in California by the United States Public Health Service, Stanford University, and the University of California under a grant from the National Canners' Association, the Canners' League of California, and the California Olive Association.

sitiveness of the muscles to the stimulation or in their resistance to fatigue (Fig. 1).

In connection with the study of the effect of vagus stimulation upon intestinal movements (1) it was found that the direct application of barium chloride to the intestines of botulism cats results in as active response as is obtained in normal animals, that cut strips of the intestines of animals which are in the last stages of *botulinus* intoxication react as normal strips when placed in warm oxygenated Ringer's solution, and that immediately after death the intestinal movements of botulism animals are the same as are observed in normal animals.

From these observations it was concluded that the disturbances in function of the muscles are not dependent upon direct damage to the muscle cell but must lie somewhere in the nerve tract by which nerve impulses are conducted to it. A series of experiments was therefore undertaken to ascertain whether there is any obstruction to the passage of nerve impulses in the nerves which supply the skeletal muscles which is analogous to that observed in the parasympathetic fibers of the autonomic nervous system.

EXPERIMENTAL.

I. Experiments to Ascertain Whether There Is Any Blocking of Impulses from the Motor Area of the Brain.

Four normal animals, two rabbits and two cats, and six botulism animals, four rabbits and two cats, were used in these experiments. All observations were made when the animals were under ether anesthesia.

Trephine openings were made in the skull and the cerebral motor areas were stimulated by the unipolar method of stimulation. The stimulating current was obtained from a DuBois-Réymond induction coil with the secondary coil kept constantly at 10 cm. Care was taken to maintain the conditions of the experiment as nearly uniform as possible in all instances.

It was found that movements of the extremities, of the muscles of the neck and trunk, and of the upper eyelids were obtained as readily in botulism animals as in normal animals.

II. Experiments to Ascertain the Threshold of Stimulation in a Skeletal Motor Nerve.

Porter (4) applied Martin's method of determining the intensity of nerve stimulus (5) to a study of the threshold of stimulation in the radial nerve for extension of the wrist. His technique was closely followed.

Decerebrated cats were used in these experiments.[1] The dorsal interosseous nerve was exposed, ligated, and cut, and a Sherrington electrode was applied distal to the point of section. Movements of the wrist were recorded by a light lever which was attached to the toes by a thread. The threshold readings were obtained in the manner described previously (1).

The results of the experiments are shown in Table I. The values obtained for normal animals and for botulism animals show little difference and both fall within the limits of normal variation recorded in Porter's series.

TABLE I.

Threshold of Stimulation in a Motor Nerve (Dorsal Interosseous Branch of Radial) for Extension of the Wrist.

Normal animals.		Animals with *botulinus* intoxication.	
Animal No.	Threshold in terms of Z units.	Animal No.	Threshold in terms of Z units.
Cat N22	2.1	Cat B11	1.8
" N23	1.8	" B14	1.2
" N25	1.8	" B16	1.1
" N26	3.9	" B19	3.4
Average.....	2.4	Average.....	1.9

Limits of variation in normal cats as established by Porter in observations upon 52 animals.

Maximum..... 6.0 Z
Minimum..... 0.6 "
Average..... 2.3 "

III. Experiments to Ascertain the Threshold of Stimulation in a Spinal Reflex.

Porter (6) also applied Martin's method to a study of the threshold of stimulation in the posterior tibial nerve for reflex flexion of the hind leg and recorded observations upon 66 animals.

Decerebrated cats were used in the experiments and the technique of Porter was closely followed. The posterior tibial nerve was exposed, isolated, ligated, and cut distal to the ligature. The proximal end was passed through a Sherrington electrode. The femur was fixed in a clamp and movements of the leg were recorded by a lever which was attached to the heel by a thread. The threshold of stimulation was obtained as in the other experiments.

[1] As in the experiments described in a previous report all operative procedures were performed under deep anesthesia.

The results of the experiments are shown in Table II. Here again, there is little difference in the values obtained for normal animals and for botulism animals, and all the values lie within the limits of normal variation in Porter's series.

TABLE II.

Threshold of Stimulation in a Motor Nerve (Posterior Tibial) for Reflex Flexion of the Posterior Extremity.

Normal animals.		Animals with *botulinus* intoxication.	
Animal No.	Threshold in terms of Z units.	Animal No.	Threshold in terms of Z units.
Cat N22	3.5	Cat B14	3.0
" N23	4.5	" B15	4.1
" N25	2.8	" B16	3.0
" N26	4.8	" B19	3.4
Average.....	3.9	Average.....	3.4

Limits of variation in normal cats as established by Porter in observations upon 66 animals.

Maximum	21.6 Z
Minimum	0.7 "
Average	5.2 "

IV. Experiments to Ascertain the Threshold of Vasomotor Reflexes from Stimulation of Afferent Nerves.

Martin and Lacey (7) have shown that:

"Reflex drop of blood pressure is the typical response in cats to sensory stimulation when the intensity of the stimulation is of the order of magnitude represented by the threshold for a spinal reflex of skeletal muscle. . . . . No difference in effect on blood-pressure was observed when different sensory nerves were stimulated. Radial, ulnar, median, sciatic, and saphenous nerves all gave pressure-drop under stimulation near the threshold."

Two series of observations were run in these experiments, one in which the animals were decerebrated and one in which they were under urethane anesthesia. Thresholds of stimulation were recorded on the sciatic, ulnar, radial, and saphenous nerves in the manner described by Martin and Lacey.

The results of the experiments are shown in Tables III and IV. In each series the average threshold of effective stimulation was approximately the same in normal and in botulism animals, and in each the values were well within the limits of normal variation which were recorded by Martin and Lacey.

TABLE III.

Threshold of Stimulation of Afferent Sensory Fibers for Depressor Effect upon the Blood Pressure. Decerebrated Cats.

Normal animals.		Animals with *botulinus* intoxication.	
Nerve tested.	Threshold in terms of Z units.	Nerve tested.	Threshold in terms of Z units
Sciatic	4.9	Sciatic	8.5
Radial	6.4	Radial	1.6
"	9.0	Saphenous	23.5
Saphenous	10.0	Ulnar	2.0
Ulnar	10.0	"	13.2
"	19.0	"	16.0
Average	9.9	Average	10.8

Parallel observations upon decerebrated normal cats recorded by Martin and Lacey.

Maximum ..... 31.5 Z
Minimum ..... 1.28 "
Average ..... 8.7 "

TABLE IV.

Threshold of Stimulation of Afferent Sensory Fibers for Reflex Depressor Effect upon the Blood Pressure. Animals under Urethane Anesthesia.

Normal animals.		Animals with *botulinus* intoxication.	
Nerve tested.	Threshold in terms of Z units.	Nerve tested.	Threshold in terms of Z units.
Sciatic	16.0	Sciatic	16.0
"	17.0	"	22.0
Ulnar	17.0	Ulnar	19.2
"	30.0	"	32.0
Radial	22.2	Radial	23.5
"	22.8	Saphenous	21.1
Saphenous	30.6	"	29.0
Average	22.2	Average	23.2

Parallel observations upon normal cats under urethane anesthesia recorded by Martin and Lacey.

Maximum ..... 37.5 Z
Minimum ..... 2.3 "
Average ..... 15.3 "

V. Experiments to Ascertain the Threshold of Stimulation of the Somatic Motor Portions of the Third, Seventh, and Eleventh Cranial Nerves in Animals Whose Parasympathetic Fibers from the Same Nerves Showed a Marked Blocking of Impulse.

In the course of the experiments to ascertain the threshold of stimulation for contraction of the pupil by stimulation of the oculomotor nerve (1) observations were made to test the intensity of stimulus necessary in normal and in botulism animals to induce initial contraction of the extrinsic muscles of the eye to rotate the eyeball. The results of the experiments are shown in Table V.

TABLE V.

*Threshold of Stimulation of the Motor Fibers of the Oculomotor Nerve for Initial Contraction of the Extrinsic Muscles of the Eye (Rotation of the Eyeball).**

Normal animals.		Animals with *botulinus* intoxication.	
Animal No.	Threshold in terms of Z units.	Animal No.	Threshold in terms of Z units.
Cat N62	8.7	Cat B64	7.0
" N58	11.6	" B53	7.6
" N61	12.6	" B51	8.4
" N54	14.3	" B67	8.7
" N56	18.2	" B66	11.0
" N52	20.0	" B68	14.0
" N60	30.0	" B71	15.0
" N50	32.6	" B73	21.0
		" B70	30.0
		" B59	35.0
Average.....	18.5	Average.....	15.8

* For comparative readings of the thresholds of the parasympathetic fibers of the oculomotor nerve in these animals see Table VI in the previous report (1).

It was found that although there is a marked increase in the threshold of stimulation of the parasympathetic fibers of the third nerve in botulism animals (1) the intensity of stimulus necessary to rotate the eyeball in botulism animals is no greater than in normal animals.

Similar observations were made of the somatic motor portion of the spinal accessory nerve whose parasympathetic fibers are carried

over to the vagus nerve, and of the facial nerve whose parasympathetic fibers constitute the chorda tympani nerve. It was found that in botulism animals whose parasympathetic fibers show a marked increase in the threshold of stimulation (1) the intensity of stimulus necessary to induce movements of the muscles of the face upon stimulation of the facial nerve, and to draw the head towards the stimulated side upon stimulation of the branch of the spinal accessory nerve which supplies the trapezius muscle, is no greater than is required to produce the same effects in normal animals.

A survey of the results of these experiments shows that the somatic motor fibers of the cranial and skeletal nerves are not affected in the manner that has been demonstrated in the parasympathetic fibers of the prosomatic and bulbosacral outflows of connector fibers in the autonomic nervous system (1) but that impulses are transmitted as readily and initial muscle response to stimulation is as active in botulism animals as in normal animals under the conditions of the experiments. In fact, nothing was found in the experiments up to this point which indicated that the nerve supply of the skeletal muscles was in any way affected by the action of the *botulinus* toxin.

When, however, an attempt was made to correlate the results of these experiments with the clinical manifestations of botulism in human beings and in animals in which the poisoning had been experimentally produced, it was found that it was still impossible to explain all the signs and symptoms of the intoxication from the data which had been collected.

For example, it had been shown that the parasympathetic fibers of the oculomotor nerve are so damaged that there is a blocking of impulse to the intrinsic muscles of the eye which would fully explain the mydriasis and loss of pupillary reflex to light, but it was also shown that there is no interference to the passage of stimuli along the somatic fibers of the oculomotor nerve in botulism animals notwithstanding the fact that in botulism there are blepharoptosis, loss of ability to accommodate for near vision, and diplopia, all signs which have been attributed to paralysis of the oculomotor nerve. Again, the experiments also showed that there is no interference to the passage of stimuli along the somatic fibers of the spinal accessory nerve, whereas in botulism it is an exceedingly characteristic manifestation that victims of the poisoning, whether human, animal, or fowl, are unable to support the head.

It was apparent that although the experiments had shown that there is no true paralysis or blocking of impulse for initial contraction of the skeletal muscles in *botulinus* intoxication, they had not furnished any explanation for the marked muscular weakness, drooping of the eyelids in human victims and limber-neck in chickens, etc., which is characteristic of botulism. Further investigation was therefore indicated, and since it had been noted clinically that sometimes a botulism victim is able to raise the eyelids or an extremity once or twice but cannot repeat the act, it was decided to investigate whether fatigue might play a part in determining the symptom-complex of *botulinus* intoxication.

VI. Experiments to Determine Whether Fatigue Is an Essential Characteristic in botulinus Intoxication.

Decerebrated cats and rabbits were used in the experiments but only the results obtained in cats are included in this report. Identical results were, however, obtained with rabbits.

The method of procedure was similar to that described by Gruber (8) although minor modifications of his technique were introduced.

After the animals had been decerebrated a slit was made in the skin of the leg over the sciatic nerve and the peroneus communis nerve was exposed and separated from the tibialis nerve. The distal end of the cut peroneus communis nerve was passed through a Sherrington shielded electrode in which were two pairs of platinum wire electrodes, the points nearest the muscle being used for determining the threshold of stimulation at intervals, and those which were distal being used to convey the fatiguing stimulations.

The tendon of the tibialis anticus muscle was then severed from its insertion and was connected to a muscle lever by means of a strong thread which passed over a pulley so placed that the pull when the muscle contracted was in the normal direction. The foot and the upper part of the leg were firmly fixed to the animal board.

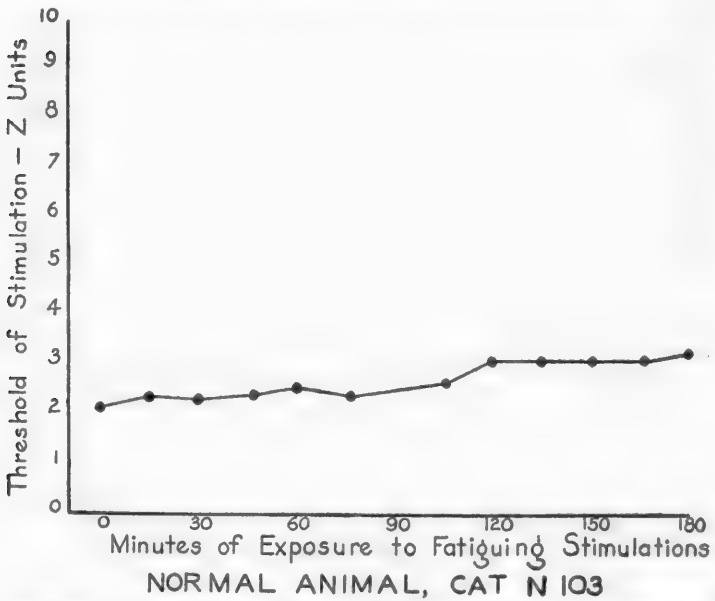
The muscle lever consisted of a pivoted metal bar which carried a writing point at one end and was provided with a spring support at the other, and the thread from the muscle tendon was attached to the lever immediately below the spring so that each contraction of the muscle would pull directly against it. The spring was adjusted so that the initial tension was approximately 100 gm., and the tension when the muscle contracted was from 120 to 150 gm.

The contractions of the muscle were recorded upon a slowly moving drum.

The fatiguing stimulations were obtained from a DuBois-Réymond induction coil with the secondary coil placed at a distance of 10 cm. from the closed position. The interruptions were obtained by a vibrating reed interrupter which was

attached to a separate cell and inserted in the primary circuit. The rate of interruption was 6 per second. The terminals from the secondary coil were attached to the distal pair of points in the Sherrington electrode.

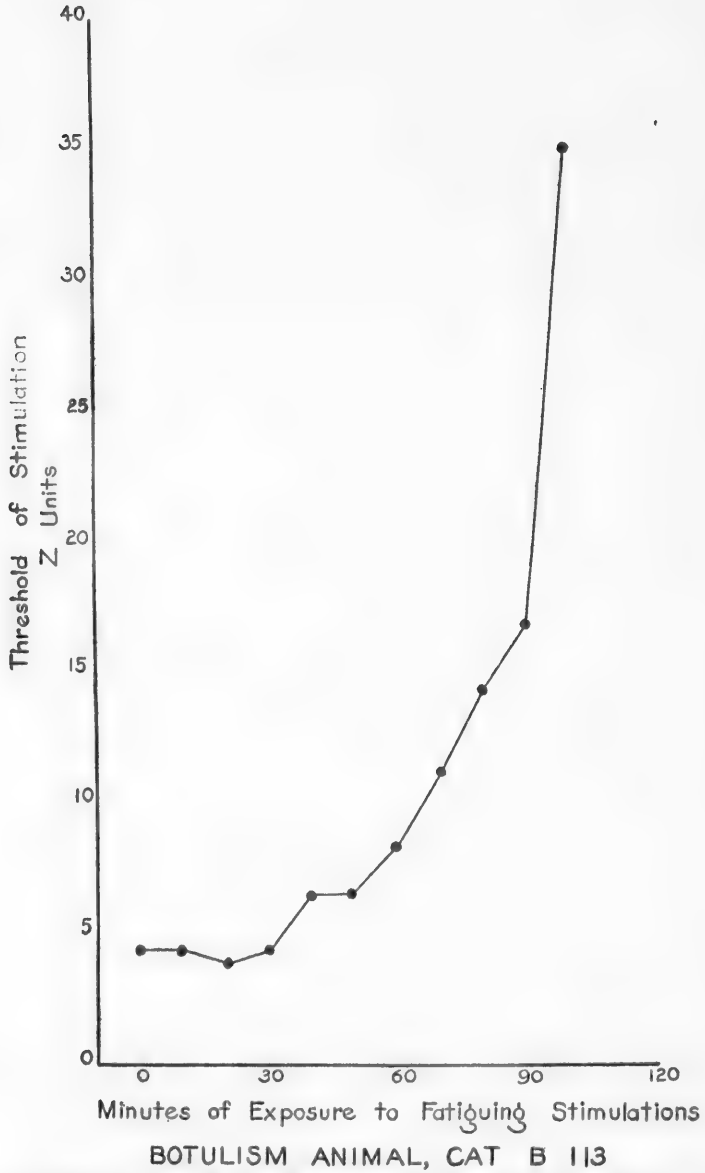
The threshold of stimulation was obtained at the beginning of the experiment and at stated intervals during the course by discontinuing the fatiguing stimulations and determining the intensity of stimulus necessary to induce initial contraction of the muscle with the calibrated inductorium as in the previous experiments, the terminals from the secondary coil of the calibrated inductorium



TEXT-FIG. 1. Rate of onset of fatigue in a normal animal as shown by the thresholds of stimulation for muscular contraction taken at regular intervals during the course of continuous fatiguing stimulations.

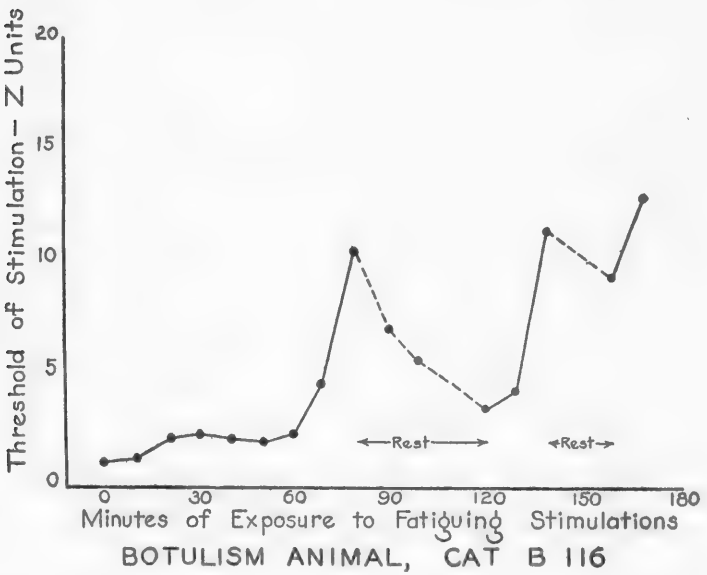
being attached to the pair of points in the electrode which were proximal to the muscle. In this way the initial threshold estimation in each experiment served as the control for the experiment as it was obviously impossible to determine the normal threshold for each animal before it was injected with the toxin.

The results of the experiments are shown in Text-figs. 1 to 4. It was found that in normal cats it is possible to apply continuous fatiguing stimulations at the rate of 6 per second for 3 hours without



TEXT-FIG. 2. Rate of onset of fatigue in a botulism animal as shown by the thresholds of stimulation for muscular contraction taken at regular intervals during the course of continuous fatiguing stimulations.

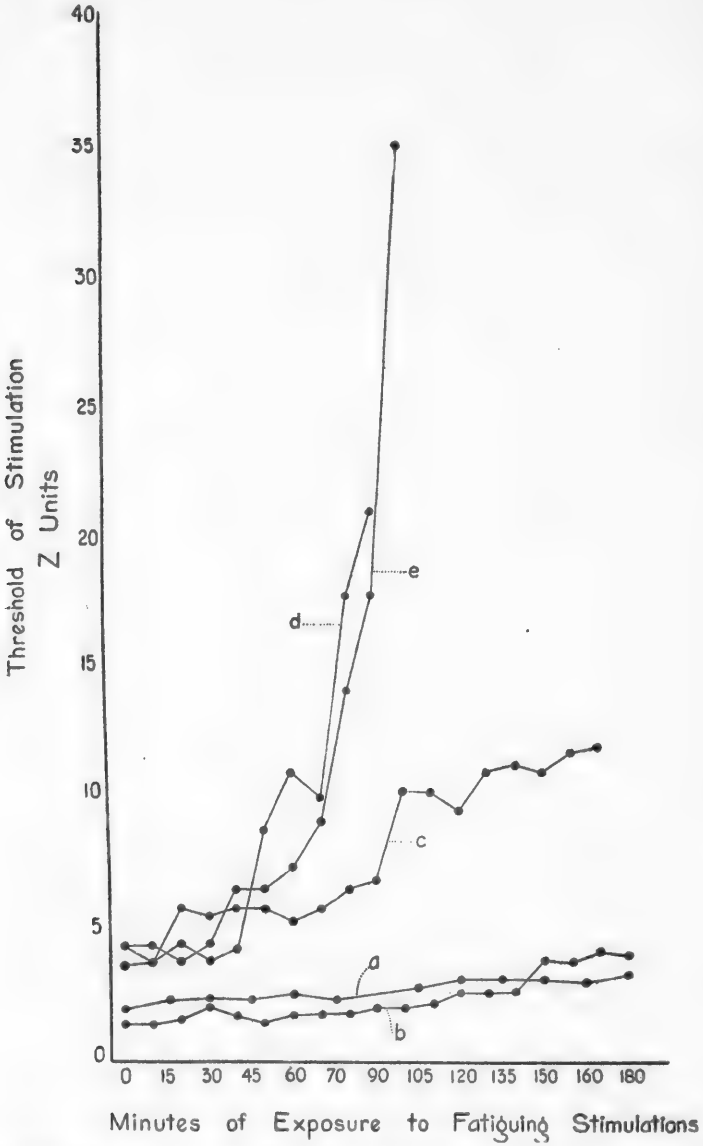
increasing the threshold of stimulation for muscular contraction (Text-fig. 1) but that in botulism animals, although the initial threshold is always within the limit of variation for normal animals, the thresholds for contractions of the muscle at intervals during the course of the fatiguing stimulations soon increase in value (Text-fig. 2). It was also found that when fatigue has developed in botulism animals, as indicated by the upward trend of the fatigue threshold curve, a



TEXT-FIG. 3. The slow recovery from fatigue and the rapid recurrence when the fatiguing stimulations are renewed.

rest of considerable duration will cause the initial threshold after the rest to approach the normal but that the rise in threshold values is much more rapid when the fatiguing stimulations are renewed (Text-fig. 3).

An attempt was then made to ascertain whether the rapidity of onset of fatigue bears any relation to the stage or intensity of the *botulinus* intoxication.



TEXT-FIG. 4. Comparison of the fatigue threshold curves obtained in a normal animal and in animals at varying intervals after the administration of equivalent amounts of *botulinus* toxin. *a*, normal animal, Cat N103; *b*, botulism animal, Cat B105, 24 hours after administration of toxin; *c*, botulism animal, Cat B106, 30 hours after administration of toxin; *d*, botulism animal, Cat B110, 40 hours after administration of toxin; *e*, botulism animal, Cat B113, 46 hours after administration of toxin.

Animals of approximately the same weight were selected and the fatigue threshold observations were made 24, 30, 40, and 46 hours, respectively, after the administration of an amount of toxin which was estimated to be sufficient to cause the death of the animals in about 48 hours. It was found that after an interval of 24 hours Cat B105 showed no signs of illness and the fatigue threshold curve showed no variation from normal, that after 30 hours Cat B106, which was weak in the hind legs but could still move about and eat, showed a gradual rise in the fatigue threshold curve, and that Cats B110 and B113, which were prostrate after 40 and 46 hours, respectively, showed a marked and rapid rise in the fatigue threshold curves (Text-fig. 4). There was little difference in the rate of the onset of fatigue in the last two animals.

DISCUSSION.

The results of the experiments which have been recorded in this and in our previous report (1) may be summarized as follows:

The action of the toxin of *Clostridium botulinum*, Type A or B, upon the nervous system is peripheral and not central. It affects the peripheral endings of the motor fibers of portions of the autonomic or involuntary nervous system and of the voluntary nervous system. It has not been ascertained whether the damage is located in the terminal nerve endings or in the myoneural junction, and the type of damage is unknown save that it is not of the nature of an organic destruction of tissue. There is no demonstrable change in the muscle cells of striated or smooth muscle.

The effect of the toxin upon the autonomic nervous system is confined to the motor and secretory fibers which constitute the parasympathetic system, described by Gaskell (2) as the prosomatic and bulbosacral outflows of connector fibers, and results in a relatively unstable blocking of nerve impulses which is manifested by disturbed function but not true paralysis of the muscles which they supply. The fibers of the sympathetic system, the thoracicolumbar outflow of Gaskell, are not affected.

The effect upon the voluntary nervous system is apparently less severe since the initial response to stimulation of the motor nerve fibers of this type is as active as is observed in normal animals, but when repeated stimulations are applied to these nerves there is a rapid and progressive fatigue which is manifested by the progressive rise in the values of the threshold of stimulation for muscular contraction

which is proportional to the intensity of the intoxication. The sensory fibers of the peripheral nerves and the reflex arcs are not affected.

In discussing the symptoms and signs of *botulinus* intoxication with reference to these findings it must be remembered that the manifestations which are typically those of botulism do not, as a rule, appear until after 18 to 24 hours have elapsed after the ingestion of the poison. The early nausea and vomiting, sometimes with diarrhea, which are observed in about one-third of the cases, are probably caused by the local action of the spoiled food in the gastrointestinal tract and not by the action of the toxin itself. This conclusion is based upon the fact that the majority of the human victims of botulism and practically all animals in which the poisoning is produced by feeding do not show signs of acute gastrointestinal disturbance, and that invariably these symptoms disappear with the onset of the true symptoms of the poisoning.

We believe that all the characteristic signs and symptoms of *botulinus* intoxication may be explained on the basis of the observations made in our experiments although it must be repeated that the actual cause has not been determined because the nature of the interference with conduction of nerve impulses has not been ascertained. In discussing them the course of a typical case of botulism will be followed and the probable cause described.

Among the earliest, and often the first indications of *botulinus* intoxication in human beings are disturbances of vision which include dimness of vision, mydriasis, loss of accommodation for near vision, diplopia, loss of pupillary reflex to light, nystagmus which is sometimes unilateral, photophobia, blepharoptosis, and vertigo.

Mydriasis, loss of accommodation, and loss of the pupillary reflex to light may all be caused by the blocking of impulses in the parasympathetic fibers of the oculomotor nerves and the photophobia is due to the persistent dilatation of the pupils as long as they are not covered by the drooping upper lid. Mydriasis is more marked because of the persisting function of the sympathetic fibers.

Vertigo is probably caused by the attempts to accommodate.

Dimness of vision may be partly due to the disturbance of accommodation but is more probably the early result of imbalance of the extrinsic muscles of the eyeball which is caused by susceptibility to fatigue in the endings[2] of the somatic

[2] Throughout this discussion where the phrase "in the nerve endings" is employed, no differentiation is implied between the anatomical terminal endings of the nerve fibers and the myoneural junction.

fibers of the third, fourth, and sixth cranial nerves which ultimately leads to diplopia and occasionally immobility of the eyeball. Blepharoptosis may be caused by susceptibility to fatigue in the somatic nerve supply of the branch of the oculomotor nerve which supplies the upper lid, and nystagmus is caused by the imbalance of the extrinsic muscles.

Closely following the disturbances of vision there is usually difficulty in talking and in swallowing, the tongue movements are sluggish, the mouth is dry, and the patient suffers from the accumulation of thick mucus in the pharynx.

Dryness of the mouth is due to an inhibition of the serous portions of the saliva caused by the blocking of impulses in the parasympathetic secretory fibers of the chorda tympani and glossopharyngeal nerves, and the accumulation of thick mucus is due to the persistent function of the sympathetic fibers which supply the salivary mucus glands.

The sluggish movements of the tongue may be due to susceptibility to fatigue in the endings of the somatic motor fibers of the glossopharyngeal and hypoglossal nerves and the sensation of enlargement of the tongue is due to the relaxed, atonic condition of its muscle fibers.

Difficulty in swallowing is partly due to the dryness of the mouth and pharynx but impaired function of the muscles of the pharynx must also play a part. This may be due to susceptibility to fatigue in the nerve endings of the somatic motor fibers of the ninth and tenth cranial nerves. That there is no true paralysis is indicated by the fact that the accumulation of mucus or attempts to swallow may induce severe strangling spells.

The earlier stages of difficulty in talking are undoubtedly due to the impaired movements of the tongue since at first the voice sounds remain but the patient cannot pronounce certain words; eventually, however, the voice sounds disappear and the patient can neither articulate nor phonate. At this stage susceptibility to fatigue in the endings of the somatic motor fibers of the vagus and possibly also of the hypoglossal nerves would explain the loss of function in the larynx. The fact that in milder cases of botulism the patient becomes progressively more husky and less easily understood if he continues to talk indicates that it is fatigue and not paralysis which is responsible for the disturbance in function.

Botulism differs from the usual types of food poisoning in that, instead of the occurrence of acute gastrointestinal disturbances, there is inhibition in the movements of the stomach and intestines so that retention of food materials in the stomach and persistent constipation are characteristic of the intoxication. It is probable that the absence of moisture and the presence of thick mucus in the bowel may play some part in causing the constipation but there is no doubt that the most important factor is the blocking of motor impulses in the parasympathetic fibers of the vagus and pelvic nerves which respectively supply the upper and lower portions of the gastrointestinal tract.

A sensation of fatigue and of general muscular weakness is an early manifestation of botulism and progressive weakness which is especially noticeable in the neck and in the extremities is characteristic of the intoxication. In cases

of mild intoxication the victims complain that they are unable to masticate their food because of weakness of the jaws and not infrequently the face has an expressionless appearance because of the relaxed tone of the facial muscles. In the typical severe case the patient lies helpless in the bed with the muscles relaxed in a manner not unlike that seen when under deep general anesthesia, but although the condition may be so severe as to simulate paralysis, it is characteristic that the deep reflexes are intact and that the patient may be able to initiate effective muscular contraction to open the eyes or raise the head or an extremity once or twice although he cannot repeat the act.

All these phenomena may be explained on the basis of susceptibility to fatigue in the nerve endings of the motor fibers which supply the skeletal muscles, and it is significant that in those portions of the body where sustained muscular action is necessary to maintain the normal position of the part—the upper eyelid in man and the head and neck in fowl and quadruped animals—failure of function in these parts occurs early in the course of the intoxication.

Inhibition of many of the secretions is also characteristic of botulism and reference has already been made to the absence of the serous portion of the saliva. It has been reported (9) that, especially in animals, there may be a salivation instead of an inhibition of saliva, but this has not been observed in any of our human cases or in our experimental animals. It has been noted in our experiments that the animals may continue to champ the jaws, apparently in the attempt to free the mouth from thick mucus, and it is our opinion that it was this and not a true salivation which was the cause of the apparent dribbling in the cases which have been reported.

It has been reported that inhibition of the secretion of urine is characteristic of botulism but we believe that this is not strictly correct. It is true that because the patient is unable to swallow the water intake is greatly restricted and the urine output is correspondingly decreased, but there is no evidence that there is cessation of kidney function. There may be retention of urine in the bladder, however, and the reason for this is suggested by the failure to induce contraction of the wall of the bladder by stimulation of the parasympathetic fibers of the pelvic nerve.

There is lack of uniformity in the reports of human victims of botulism in as far as the secretion of the sweat glands is concerned. In the earlier literature it was stated that there is inhibition of sweat secretion and that the skin becomes hard and dry but in our series of cases there have been a few instances in which the patients were bathed in perspiration late in the course of the intoxication. It is impossible to correlate these observations with the results of the experiments since, anatomically, the sweat glands obtain their nerve supply from the sympathetic system and our experiments show no effect of *botulinus* toxin upon the sympathetic fibers. However, it is of interest that in the experiments with acetyl choline Dale (10) observed that the effect upon the sweat glands was one of the chief exceptions to the general parasympathetic action of the drug.

The initial slow pulse rate in botulism and the rapid pulse which is characteristic of the later stages of the intoxication are fully explained by the experiments upon the cats in various stages of the poisoning. The experiments showed that in the first 24 hours of the intoxication there is no disturbance of the transmission of inhibiting impulses through the vagus nerves but that after that time the threshold of stimulation for inhibition of the heart rate is greatly increased.

When death occurs from cardiac failure the heart muscle is found to be relaxed and flabby and it is probable that it ceased its action because of fatigue.

In the early stages of botulism there is no disturbance of respiration beyond what may be explained by the psychic response of the victim who is often excited and apprehensive as to the outcome of his illness, but in the later stages of the intoxication breathing becomes labored and all the accessory muscles of respiration are brought into play. Toward the end in fatal cases there may be indications of asphyxia and sometimes there is Cheyne-Stokes respiration, both dependent upon the combination of disturbed respiration and impaired heart action.

The respiratory distress may be explained by a loss of the controlling influence of the vagus nerves upon respiratory movements, and when death occurs from respiratory failure it is undoubtedly due to the fact that because of a susceptibility to fatigue in the nerve endings which supply them, the accessory muscles of respiration are unable to maintain their function. Fischer (11) has shown that the diaphragm ceases to function before the accessory muscles of the chest.

There is no indication that the subnormal temperature in botulism is in any way directly dependent upon disturbance of the nervous mechanism of temperature control since when patients who have shown the typical subnormal temperature throughout the course of the intoxication develop insufflation bronchopneumonia the temperature promptly rises. It is probable that the subnormal temperature is dependent upon the condition of general muscular relaxation and inactivity although it is possible that there may also be some underlying factor, perhaps some insufficiency of the ductless glands, which is the primary cause of the disturbance of function in the nerve endings as well as of the lowered body metabolism.

The maintenance of normal blood pressure, the persistence of secretion of the mucus portion of the saliva, and the extreme dilatation of the pupils suggest that, as in the experiments, the sympathetic system is not involved; and the absence of any demonstrable variations from normal in the cerebrospinal fluid may be construed as indicating that there is no gross change in the tissues of the central nervous system.

Clinically, as well as in the experiments, there is no disturbance of sensory function in botulism, and it is characteristic of the intoxication that the victims show no disturbance of mentality so long as the functions of circulation and respiration are maintained.

It is not the purpose of these reports to discuss the treatment of botulism, but there is one group of observations which should be mentioned in connection with the administration of *botulinus* antitoxin.

In a previous report (12) it was noted that when guinea pigs are injected with a sufficient amount of toxin to cause their death in about 48 hours, the administration of antitoxin within 24 hours after the toxin will enable them to survive, but that if the antitoxin is delayed beyond that interval they will succumb. The results of the experiments[3] outlined in a previous report (1) and in Text-fig. 4 of this report are of interest in this connection because they show that there is no demonstrable interference with the conduction of impulses in the parasympathetic fibers and no increased susceptibility to fatigue in the somatic motor nerves within 24 hours in *botulinus* cats but that in 30 hours disturbances in function can be demonstrated and that after that time they are marked.

There can be no doubt that the peculiar relationship which exists between the results of these three sets of experiments is not merely coincidence. The indications are plain that up to a certain time, under the conditions of the experiments 24 hours, the *botulinus* toxin is not so bound to the tissues that disturbances in function can be demonstrated by the methods used in the experiments, and that during this interval it is possible to counteract its influence by the administration of antitoxin, but that after that interval has elapsed the toxin is so bound with the tissues that disturbances in function can be demonstrated and that when this is true the administration of antitoxin does not neutralize its effect. This corresponds with what was observed clinically because in the experiments the animals had not shown any definite indication of illness up to 24 hours after the administration of the toxin. It also suggests a reasonable explanation for the fact that in the treatment of human victims of botulism the administration of antitoxin after the onset of symptoms has not proved to be of any value.

The conception that *botulinus* toxin exerts its action peripherally and not directly upon the central nervous system was first expressed in 1877 by Pürckhauer (13) who based his conclusions upon clinical observation. More recently Schübel (14) has advanced a similar conclusion, his preliminary report being made within a few days of our

[3] Dickson and Shevky (1), Table II.

own (15). His experiments were performed upon frogs and certain invertebrate forms and he found that frog muscle does not undergo any change after a chronic intoxication of several weeks, and that there is a peripheral action upon the end-plates of the nerves which he describes as simulating that produced by curare. In addition to the peripheral action of the *botulinus* toxin Schübel describes degeneration in the spinal cord of frogs, a process which we have been unable to demonstrate in our experiments with warm blooded animals.

CONCLUSIONS.

1. In addition to the effect upon the fibers of the parasympathetic nervous system which was described in a previous report (1), the toxin of *Clostridium botulinum*, Types A and B, exerts an influence upon the endings of the motor fibers of the voluntary nervous system which leads to a marked susceptibility to fatigue. It has not been determined whether the damage is in the anatomical nerve endings of the somatic motor nerve fibers or upon the myoneural junction, but it is not of the nature of an organic destruction of tissue.

2. There is no effect upon the sensory fibers of the peripheral nerves.

3. The muscle cells of the smooth and striated muscles are not affected.

4. The disturbances in function which have been demonstrated in the voluntary and involuntary nervous systems fully explain the characteristic signs and symptoms of *botulinus* intoxication.

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EXPLANATION OF PLATE 24.

FIG. 1. Tracings showing the rate of onset of fatigue in excised thigh muscles of two guinea pigs of equal weight, one of which was in the later stages of *botulinus* intoxication (Type A). Stimulations were applied directly to the muscles at the rate of 40 per minute. The break in the indicator record denotes a period of rest of 5 minutes. The muscles were suspended in aerated modified Locke's solution which was maintained at body temperature.

FIG. 1.

(Dickson and Shevky: Botulism. II.)

STUDIES BASED ON A MALIGNANT TUMOR OF THE RABBIT.

V. Metastases.

Part 1. Description of the Lesions with Especial Reference to Their Occurrence and Distribution.

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Plates 25 to 29.

(Received for publication, May 25, 1923.)

In previous reports (1, 2), dealing with phenomena of tumor growth and the clinical effects observed in tumor-bearing animals, reference was made to the frequent occurrence of metastases following intratesticular transplantation and their absence after other methods of inoculation, and it was pointed out that this was one of the outstanding differences in the results obtained by the use of various methods of transplantation. It was further noted that no constant relationship existed between the rate of growth or the size attained by the primary tumor and the occurrence of metastases in distant organs, but that the development of metastases and the malignancy displayed by the tumor were more closely related to persistence of growth or the ability of the animal to confine the growth to a given area, and hence to the function of animal resistance.

If one may consider metastases from this point of view, a study of the phenomena of metastasis of a given tumor in a large series of animals, should not only throw some light upon those conditions which contribute to or prevent the occurrence of metastases but upon the more fundamental problem of the nature and mode of operation of animal resistance. Such a study has been undertaken, and the results, in as far as they pertain to the occurrence or non-occurrence of metastases, will be reported in this and the two following papers.

On account of the scope of the investigation and the volume of the material used, it will be necessary to divide the report dealing with metastases into three parts. The first two papers will be devoted to a description of the lesions and the circumstances of their occurrence as a further contribution to the picture of the disease produced by this tumor and as a basis for the discussion of factors which influence the occurrence and distribution of metastases. This last phase of the subject will be reserved for Part 3 of the paper.

Methods and Material.

The material upon which this study is based consists of a series of 191 rabbits from the first twenty generations of transplantation in which the phenomena of metastasis were studied in relation to the growth of the primary tumor, the clinical course of the disease, the occurrence of changes in other organs and tissues, and a number of other factors such as the age and breed of the animal, the generation of the transplant, and the season at which the inoculations were made, all of which are known to exercise some influence upon the course of the disease. For the most part, the animals were derived from experiments which were conducted primarily for the study of the disease as a whole, and hence from the single point of view of metastases, the material is not so well distributed as might be desired, but all animals have been excluded where any objection might be raised to their use. The animals fall into two general groups: first, animals killed at regular intervals of from 1 to 4 weeks after inoculation, and second, animals that died or were killed at various periods during the progress or resolution of the growth or after apparent recovery had taken place, the time ranging from 2 weeks to 7 months from the date of inoculation. For statistical purposes, however, the time limits have been fixed at between 3 and 28 weeks.

All animals received a unilateral testicular inoculation, and with two exceptions, no experimental procedure was employed which in itself might influence the course of events. The exceptions mentioned included one group of four rabbits which had one testicle removed before inoculation and a group of five rabbits that were inoculated with material that had been subjected to repeated freezing and thawing with a consequent prolongation of 2 to 3 weeks in the period of incubation. Otherwise, the variables which figured in the results were those of age, breed, length of survival, and such unavoidable differences as might exist in the quality of material used for the inoculation of different series of animals and the time (season) at which the experiments were carried out.

In reporting the results of this investigation, estimates of incidence and distribution will be based upon metastases which could be detected clinically or at autopsy or to gross as distinguished from microscopic lesions. In estimating the actual incidence of metastases, all animals showing active, regressing, or healed

lesions have been regarded as positive, but in classifying lesions on a time basis, it has been necessary to disregard clinical observations as to the time of occurrence of certain groups of metastases and to eliminate healed lesions, since these cannot be properly correlated with autopsy observations. The time given in such classifications unless otherwise indicated is that at which the postmortem examination was made.

The method of presentation employed is based upon the animal as the prime factor in determining the course of the disease. Lesions are described from the point of view of the peculiarities of the growth and the reaction which occurs in individual organs and tissues, and the circumstances of their occurrence are analyzed with reference to time and to the character and progress of the disease, the object being to bring phenomena of metastasis into as close relationship as possible with those conditions which determine the course of the disease.

Many of the facts in regard to metastases are represented graphically, and for this purpose, two simple methods of charting have been used, both of which are intended to represent relative rather than absolute values. The animals studied are immediately divisible into two main groups, positives and negatives. While we are equally concerned with both groups, our interest is at present centered upon those animals in which metastases occurred. Hence in order to avoid undue distortion of the picture presented, it is necessary to eliminate negative animals and to record the results of incidence of different classes of metastases upon a relative basis.

Moreover, the mere presence of metastatic growths may in itself be misleading and the further consideration of the number of foci affected has been introduced in order to give the added idea of the severity of disease as expressed in terms of the incidence and distribution of metastases in any group of animals or tissues. In like manner, the general curve of metastatic involvement has been used in many places as a background for comparison with the curves of individual organs in order to preserve the idea of the relation of metastases in individual organs to the disease as a whole.

Incidence and Distribution of Secondary Growths in General.

In taking up the subject of secondary growths arising from this tumor, it seems well to emphasize the fact that the tumor under consideration has at all times exhibited a tendency to a diffuse growth with a perivascular arrangement of cells and that invasion of blood vessels and lymphatics has occurred almost from the beginning of the growth or within 7 to 10 days after inoculation. It is obvious, therefore, that some dissemination of cells must have occurred in practically all animals with actively growing primary tumors, and yet, among the animals studied, secondary tumors were found in only 114, while the

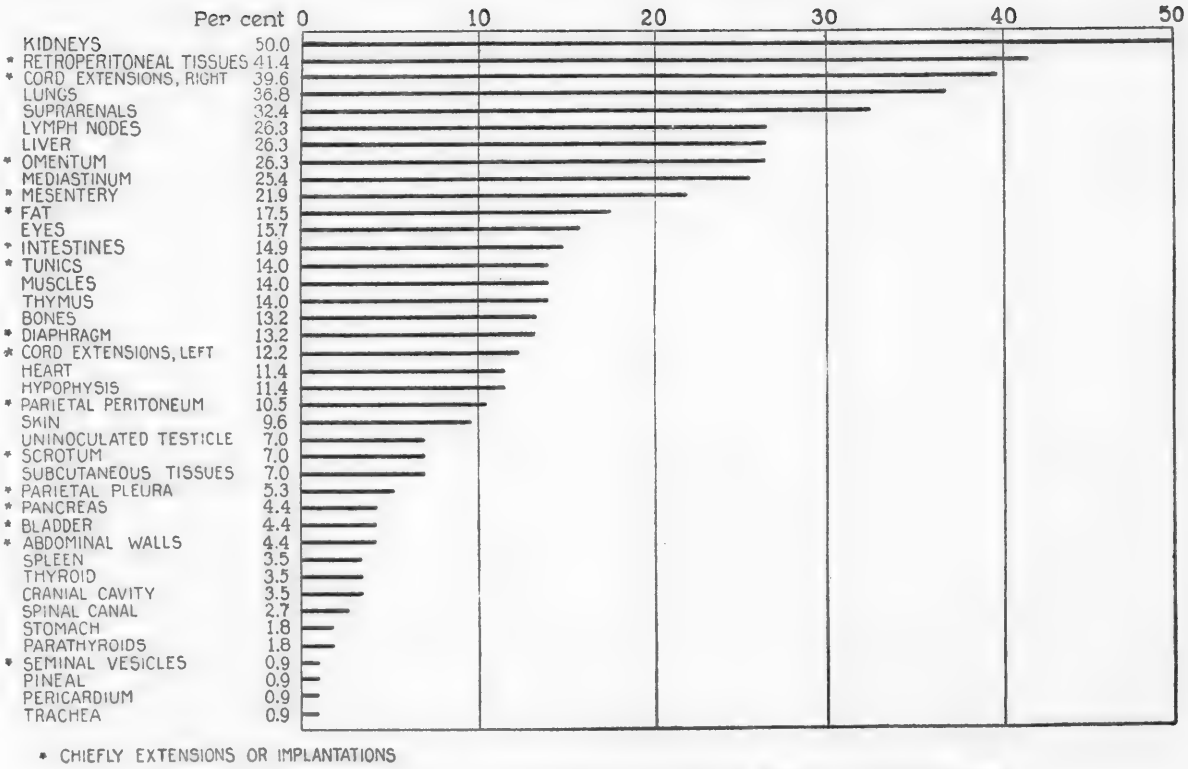
remaining 77 animals showed no visible extension of the growth beyond the body of the inoculated testicle. It would appear from this, that dissemination of tumor cells does not necessarily imply the development of gross lesions, and this fact is of the foremost importance, since in studying phenomena of metastasis, one is concerned fully as much with the absence of metastases as with their presence.

For convenience, the tumors which developed from a primary growth in the testicle have been divided into three classes, (1) local or regional extensions, (2) implantations on serous surfaces, and (3) metastases to distant parts of the body arising by way of the blood vessels or lymphatics. This grouping is intended primarily to indicate degrees of independence and distribution rather than the exact mode of origin, which in reality is one of the problems with which we are concerned.

The mean incidence of secondary growths for the entire group of animals was 60 per cent, and at one time or another metastases occurred in nearly all parts of the body, as shown in Text-fig. 1, which gives the relative frequency of involvement of various organs and tissues in 114 animals. There were great variations, however, in both the incidence and distribution of metastases, and, while it is not intended to take up this phase of the subject in detail, the fact should be noted that at different times and in different series of animals, the incidence of metastases varied between a minimum of about 25 per cent and a maximum of 100 per cent and that the distribution of the lesions was subject to equally marked variations. These features of the subject will be discussed when the factors are considered which have influenced the growth and malignancy of the tumor during the course of transplantation.

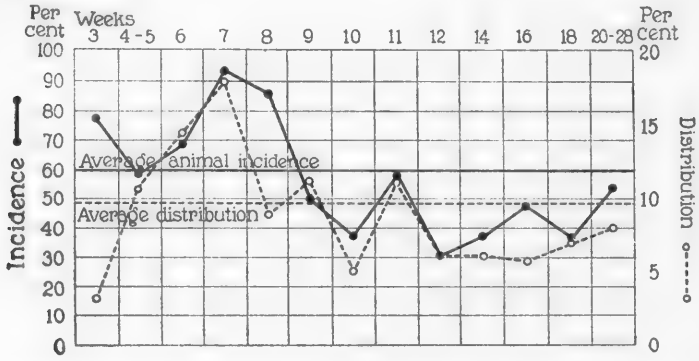
Gross lesions were found as early as 2 weeks after inoculation, and in some instances, actively growing metastases were still present in animals killed as late as 7 months after inoculation. The curve of incidence of all classes of lesions as determined by postmortem examination at periods of from 3 to 28 weeks after inoculation is given in Text-fig. 2. If this chart is considered with reference to the development of the primary tumor and the subsequent course of the disease in different classes of animals, it possesses a significance which is not otherwise apparent, and since this form of chart will be used with reference to metastases in individual organs, it may be analyzed in some detail.

In the first place, two conditions are represented. The upper curve indicates the proportion of the animals examined at any time in which secondary growths were found, and may be spoken of as the curve of animal incidence. The lower curve represents the relative number of foci involved as compared with a theoretical possible involvement which was determined by the distribution of metastases in all animals examined thus far, and represents both the extent and distribution of lesions. In order to facilitate comparison of these two curves, the scale of the second curve has been multiplied fivefold.



TEXT-FIG. 1. Relative incidence of secondary tumor growths in different organs and tissues.

A consideration of the conditions found at 3 weeks will show that a great majority of the animals (77 per cent) showed secondary growths but few foci were affected (3.08 per cent). From the 3rd to the 5th weeks, corresponding with the time during which many animals show either an arrest or regression of the primary tumor, fewer animals showed metastases, but those in which metastases were found, showed a decided increase in distribution or the number of organs affected. From this point onward, the two lines proceed almost parallel with a rapid increase in both the incidence and distribution of metastases until the maximum height is reached at the 7th week. There is then a marked reduction in both curves extending over a period of several weeks followed by a slight rise with a second drop after which the changes that occur are of minor degree and affect animal incidence rather than the distribution of metastases, but there is a distinct upward trend in the curve of incidence.



TEXT-FIG. 2. Relative incidence and distribution of metastases, plotted with reference to time.

These changes are all reflected in the course of the disease. The initial drop in the curve of incidence indicates that early metastases may undergo resolution in some animals at the same time that retrogressive changes take place in the primary tumor. The rapid rise in the curves between the 4th and 7th weeks corresponds with a renewal of activity in the primary tumor and the appearance of cases of fulminating malignancy. The apparent increase in the curve of incidence is doubtless somewhat exaggerated owing to a preponderance of deaths at this particular time but this is a characteristic feature of the disease. The drop which occurs from the 7th to the 10th weeks is due in part to elimination by death and in part to recovery of a second group of animals. The wide discrepancy between incidence and distribution as shown at the 8th and 9th weeks is almost a repetition of the condition at the 3rd and 4th weeks and is doubtless attributable to the same causes. Subsequent elevations in the curve of incidence are attributable to recru-

descence of activity and more slowly progressive types of disease which, as may be seen, mature at intervals of approximately 4 weeks. The flattening out of the curve of distribution which occurs during the latter half of the period of observation is of especial importance since, as will be shown later, the maintenance of this particular level is due to the almost constant involvement of one group of organs and of conditions which suggest the development of a selective distribution of cells or an immunity in certain tissues which is not shared by others.

The facts which have been brought out show the general tendencies which exist with reference to the occurrence of secondary growths as a whole. It has been found, however, that the conditions which favor the development of metastases are not the same for all organs or tissues, and in order to obtain a clearer conception of the underlying causes for these differences it will be necessary to consider the lesions of different organs individually.

Local or Regional Extensions.

While in many animals, the primary tumor was at all times confined within the testicle, the growth not infrequently invaded the tunics and spread to adjacent tissues or extended outward along the lymphatics and blood vessels either in the form of fairly discrete nodules connected by more or less distinct lines of tumor growth, or as masses continuous with the primary tumor. The resulting lesions, therefore, were of two kinds, but with no sharp line of distinction between them. The picture presented varied in different animals. In some, there were small nodular masses on the outer surface of the tunics, and occasionally the growth involved the scrotum as well. More often there were discrete nodules distributed along the cord, or the growth extended upward into the abdominal cavity in the form of large, irregular masses which not infrequently involved the tissues about the inguinal canal and the lower abdominal and pelvic regions. Examples of the conditions present in cases of this kind may be seen in Figs. 1 to 3, which show varying degrees of the processes described.

These outgrowths from the primary tumor were at times very limited in extent or were confined to the lower abdominal and pelvic regions. As a rule, however, the growth continued upward in the retroperitoneal tissues along the midline of the body or along the outer margins of the lumbar muscles (Figs. 1 to 3). In these cases, the direct connection with the primary tumor was usually lost. The growth assumed the form of discrete nodules distributed along the line of the lymphatics

with numerous metastases in the retroperitoneal lymph nodes and in the perirenal tissues, and not infrequently, this chain of lesions extended upward in the midline to the base of the neck with the formation of metastases in the retropleural tissues and in the mediastinum.

Growths of the several types described were of frequent occurrence as may be seen by reference to Text-fig. 1. Out of a total of 114 animals, there were only 14, or 12.3 per cent, in which the only secondary growths were confined to the tissues immediately surrounding the primary tumor. Extensions up the cord of the inoculated testicle occurred, however, in 39.6 per cent of the animals, and it is important to note that the retroperitoneal tissues were involved with even greater frequency (41.4 per cent), while secondary growths were comparatively rare in either the tunics or the scrotum.

Extension of the primary tumor up the cord and the development of isolated nodules in the epididymis and cord were usually the first clinical signs of malignancy and were frequently recognizable by the end of the 3rd or 4th week after inoculation. In like manner, metastases to the retroperitoneal tissues occurred as early as 2 to 3 weeks after inoculation, or at about the same time and with the same frequency as pulmonary metastases, which shows not only that the cells began to be disseminated at a very early period but presents a striking parallelism in the initiation of metastases by way of the blood vessels and lymphatics.

Lesions of this class rarely gave rise to serious disturbances. In several animals, there was obstruction to the right ureter with the production of a hydronephrosis, and in one instance, a growth in the pelvis caused complete obstruction at the neck of the bladder.

Implantations.

Invasion of the tunics and extension of the primary tumor into the lower abdominal cavity were productive of conditions which might be regarded as peculiarly favorable to the development of implantation metastases but lesions of this class were by no means so frequent as might have been expected. In fact, they were less frequent than metastases to the retroperitoneal tissues or to such organs as the lungs and the kidneys, where the distribution of tumor cells was effected either by the lymphatics or the blood stream (Text-fig. 1).

In some instances, there were innumerable masses of tumor growth distributed through all parts of the abdominal cavity, as in Fig. 1, but these cases were comparatively rare. As a rule, there were only a few lesions present, and while some of them were as large as 8 to 10 cm. in their greatest diameter, they were usually small and rarely exceeded 1 to 2 cm. in diameter.

These growths occurred most often in the omentum and mesentery but were almost as frequent over the surface of the intestine and diaphragm together with

adjacent parts of the abdominal wall. The stomach and bladder were rarely affected and metastases were less frequent on the small intestine than on the cecum and colon. The surface of the spleen was affected in only one animal, and as far as could be determined, there were no implantation metastases on the liver.

In like manner, metastases were less frequent over the lateral and ventral surfaces of the parietal peritoneum than in the pelvic and diaphragmatic pouches, the flanks, or the lumbar regions. Another peculiarity of these lesions was a tendency to localization along the course of blood vessels and lymphatics, as may be seen in Fig. 1, which suggests that in reality some of the lesions classed as implantation metastases may have arisen from cells within blood or lymph vessels. This applies especially to mesenteric metastases.

There were a few implantation metastases on the parietal pleura, but again these lesions were rare as compared with the incidence of metastases in the lungs which involved the pleural surfaces. The evidence available, therefore, would indicate that successful implantation of tumor cells on serous surfaces was accomplished with difficulty, and that the hindrance to growth was much greater than that encountered in either the lymphatics or the blood vessels.

Although implantation metastases were of frequent occurrence and occasionally reached a very large size, there was only one instance in which death could be attributed to a growth of this kind. In this animal, an unusually large tumor developed in the mesentery and eventually gave rise to an intestinal infarction.

Metastases in Distant Parts of the Body.

By far the greatest number of secondary tumors, and those of chief importance, were located in parts of the body which were inaccessible to cells from the primary tumor except by passage through lymphatics or blood vessels. Lesions of this class occurred in 100, or 52.3 per cent, of the 191 rabbits studied, while their relative frequency in animals with secondary growths was 87.7 per cent.

Metastases of this class occurred in nearly all parts of the body, as may be seen by reference to Figs. 1 to 4 and Text-fig. 1 which gives the distribution according to incidence in different organs or tissues. The sites of greatest frequency were the kidneys, the lungs, the suprarenals, the lymph nodes, the liver, the eyes, the muscles, the bones, the heart, and the tissues of the mediastinum. In contrast with the high incidence shown by the organs enumerated, it will be noted that metastases occurred very rarely in certain other organs such

as the uninoculated testicle, the spleen, and the thyroid, while no metastases were found in such organs as the brain and spinal cord or the salivary glands.

In like manner, the lesions were confined almost entirely to the trunk, the head and neck, and the proximal segments of the extremities; as far as is known, there were no metastases in the ears or tongue or in the feet. In general, it appeared that the frequency of metastases diminished with the distance from the central axis of the body, and this was not entirely attributable to the nature of the tissues composing these parts, since metastases were comparatively frequent in tissues of the same order but nearer the body axis.

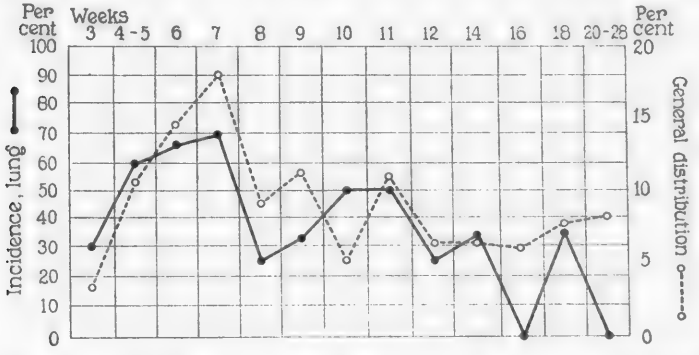
On account of the importance of this group of metastases, it will be necessary to consider them in greater detail than those of the two preceding groups. In describing these lesions, emphasis will be placed upon the time relations of metastases in different organs and tissues both with reference to one another and to the course of the disease. The order in which different organs or tissues will be taken up has been determined by the relationships which obtain as expressed in the circumstances under which metastases occurred.

Lungs.—Pulmonary metastases were usually small discrete nodules of a grayish white or opalescent appearance; they were usually firm and rarely showed either hemorrhage or necrosis. They were more numerous in the lower than the upper lobes and exhibited a tendency to localize in the pleura (Fig. 3). Occasionally, the lungs were thickly studded with these nodules, but as a rule, they were few in number and widely scattered. Among animals with metastases, the lungs were affected in 36.8 per cent. The time relations of these lesions with reference to the progress of the disease are especially interesting and can be shown best by means of a chart which records the relative incidence of pulmonary metastases in different groups of animals showing secondary growths, autopsied at from 3 to 28 weeks after inoculation (Text-fig. 3).

In general, the chart shows a three period elevation with a decrease in the extent of each succeeding rise which gives the curve a downward slope from the point of greatest elevation between the 4th and 7th weeks. These features of the curve may be emphasized, since they are typical of a condition which obtained with a number of organs while in striking contrast with the curves of others.

The actual time of occurrence of metastases in the lungs or other organs can be stated only in a general way. It may be seen from the relative position of the two curves in Text-fig. 3 that lung metastases occurred ahead of the general involvement of other organs. The earliest pulmonary metastases were noted 2 weeks after

inoculation. At the end of the 3rd week, they were more frequent than metastases in any other part of the body, except in the immediate vicinity of the primary growth. Moreover, while there was a reduction in relative frequency during the later periods of observation, pulmonary metastases were still comparatively frequent in all groups of animals up to the 16th week. After this, they were of sporadic occurrence, and the cases observed were without exception among animals of the first few generations in which the disease progressed much more slowly than at present. This fact should be mentioned because an effect of the same kind has been introduced into several other curves, notably those of the kidney and liver. In reality, there is considerable evidence to show that pulmonary metastasis were of more frequent occurrence than the actual figures would indicate and that they not infrequently underwent spontaneous resolution without leaving any gross evidence of their previous existence. The features of pulmonary metastases which



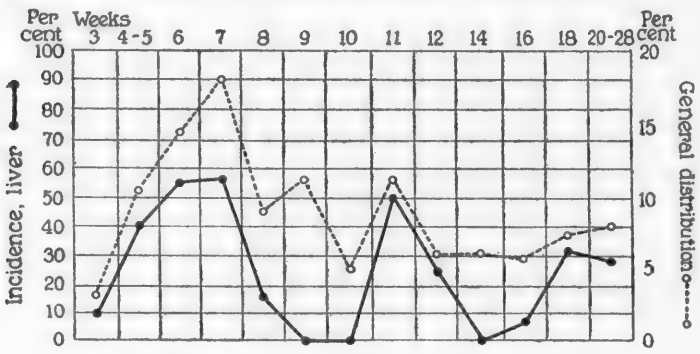
TEXT-FIG. 3. Relative incidence of lung metastases as compared with general distribution of secondary growths, at different intervals of time after inoculation.

are of especial interest are their early occurrence and relatively high frequency during the very early stages of the disease, as contrasted with the occurrence of few lesions of small size, and their tendency to diminish in frequency during the later periods of observation. After the first few weeks, during which they occurred in all classes of animals, they were in general lesions of severe rather than of mild forms of disease.

Liver.—The growth in the liver varied greatly in different animals. In most instances, there were a few large or medium sized nodules measuring from 0.5 to as much as 2.0 cm. in diameter. Individual lesions were of a soft, medullary consistency and not infrequently showed hemorrhage and necrosis, but they were rarely enclosed by a connective tissue capsule, except in animals with a long standing tumor growth. In a few instances, partially or completely healed lesions were found.

Not infrequently the liver showed a most extensive metastatic involvement with innumerable large or small nodules distributed through all parts of the organ as shown in Figs. 5 and 6. These conditions corresponded with differences in the rapidity with which the disease progressed and from this standpoint are illustrative of similar differences which existed in other organs, especially the kidneys. The growth in these cases was associated with a marked enlargement of the liver. The weight of the organ per kilo of body weight was at times increased as much as two or three times that of the normal animal. This change was also proportional to the rapidity with which the disease progressed.

The curve of incidence of liver metastases (Text-fig. 4) shows a clearly defined division into three periods, and each period of increase lies within and parallel with the curve of general distribution. The notable differences between the two curves are at those points where liver metastases were at their lowest level, indicating that



TEXT-FIG. 4. Relative incidence of liver metastases.

the liver contributed very little to the general picture of the disease at these times. The periods of greatest frequency of liver metastases were again periods of widespread systemic involvement. The terminal rise might appear to contradict this statement, but the animals responsible for this feature of the curve all came from early generations, as was noted with the lungs.

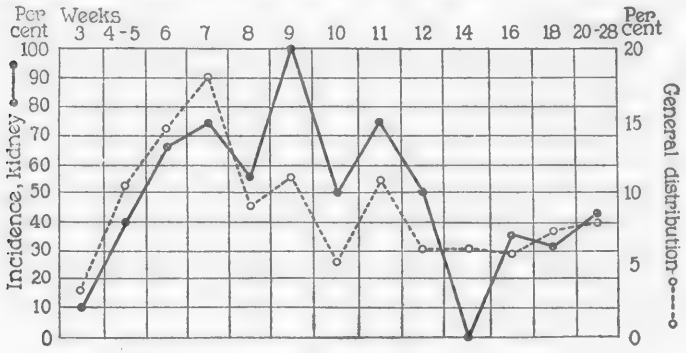
In contrast with pulmonary metastases, those of the liver were much larger, and more numerous, suggesting that opportunities for growth were better than in the lungs but that the organ was in general less accessible to tumor cells and hence was involved only at times when cell distribution was at its optimum.

Kidneys.—The kidneys were not only the most frequent sites of metastases but they also showed the greatest degree of involvement. As a rule, both kidneys were affected, and while in some instances there were only a few large or small nodules, comparable in all respects to those of the liver, the surface of the organ was more often completely obscured by an irregular nodular mass of tumor growth (Figs.

2 and 3). Metastases were located primarily in the cortex and in the subcapsular tissues; a few lesions developed in the pelvis of the kidney, but the medulla was practically free, except as it became involved by extension of the growth from other parts of the kidney or from surrounding tissues. As a rule, the growth developed in the outer portions of the cortex and extended outward rather than inward.

In instances of slowly progressive disease, the individual lesions were usually large, and not infrequently there was a marked thickening of the capsule. In more rapidly progressing cases, the lesions were smaller but still numerous. These lesions were frequently hemorrhagic in character and gave rise to profuse subcapsular hemorrhages.

Encapsulated, necrotic, and completely healed metastases were found in a considerable number of animals. In these cases, there were usually only a few lesions, but one animal was encountered with almost complete healing of what appeared to have been an extensive involvement of both kidneys.



TEXT-FIG. 5. Relative incidence of kidney metastases.

The form of the curve of renal metastases (Text-fig. 5) is different from that of either the lungs or the liver. During the earlier phases, it conforms more nearly to that of general metastatic involvement than does the curve of any other organ. This might be expected on account of the high incidence of renal metastases, but it is important to note that the maximum incidence is not reached until the 9th week, or at a time when metastases in most organs have begun to decline or have already reached a low level. Moreover, a high incidence of renal metastasis was maintained during the first 12 weeks, the curve from the 8th to the 12th weeks exceeding but conforming to that of distribution, indicating that a large proportion of the metastases found during this period was referable to the kidneys. At the 14th week, the curve reaches the base line but again rises with the distribution curve. This final rise, as with the two preceding groups of metastases, was due largely to animals from early generations.

The most significant feature of the curve of renal metastases is its sustained elevation and in this respect it resembles the curve of pulmonary metastasis. Its trend is upward, however, at a time when pulmonary lesions are diminishing, and this difference is even more striking in comparison with the course of events in the liver. These characteristics of the curve, together with the extensive growth of renal metastases, suggest two things: first, accessibility to tumor cells, and second, adaptation to the growth of metastases, or its converse, low resistance to tumor growth.

Heart.—Comparatively few animals showed metastases in the heart. The lesions were located chiefly in the tissues about the coronary sinuses and vessels or were distributed diffusely through the myocardium. In a few instances, masses of tumor cells were found in the right auricle or ventricle, apparently free or attached to the walls of the heart by a slender pedicle.

The metastases comprising this group varied from minute points to nodules measuring as much as 1 cm. in diameter. The majority of the metastases in the myocardium and many of those along the course of the coronary vessels were extremely small, semitranslucent masses of a firm consistency and showed no gross evidence of hemorrhage or necrosis, and apparently none of them underwent spontaneous resolution.

The larger nodules, on the other hand, were of a medullary character and showed extensive retrogressive changes. Metastases of this type were confined to the epicardial tissues about the base of the heart and apparently represented lesions of a different order from those in the myocardium. In one instance, a lesion of this character was found in the parietal pericardium.

The extent of the cardiac involvement was usually slight, but in a few animals with an unusually malignant disease, there were innumerable foci of tumor growth distributed through all parts of the heart.

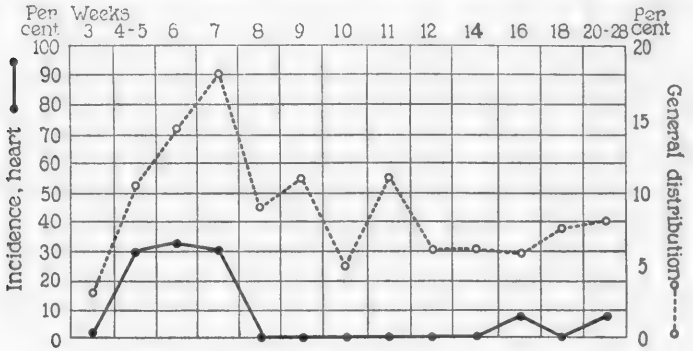
The most significant feature of the occurrence of cardiac metastases was that they were confined almost exclusively to animals with extremely malignant growths. This is indicated by the relatively high point reached in the curve of incidence at 4 weeks and the absence of cardiac metastases after the 7th week (Text-fig. 6). In fact, the cases of most extreme cardiac involvement occurred in animals that showed marked debility 4 weeks after inoculation. The liver shown in Fig. 6 came from one of these animals and metastases were almost as numerous in the myocardium. The few exceptions to this rule occurred in animals with marked mediastinal involvement, and the cardiac lesions present were of the same general character as those in the mediastinum and were confined to the epicardial tissues about the base of the heart. A few lesions of this kind were found in animals autopsied several months after inoculation.

Suprarenals.—Metastases in the suprarenals were of frequent occurrence. Of the 114 animals showing metastases, 37 showed metastatic growths in the suprarenals. In 10 instances, both suprarenals were involved, while the right was affected in 12 and the left in 15 animals.

The growth usually arose in the cortex at one or several foci. These tended to coalesce, forming a single mass which occupied the center of the gland and was usually surrounded by a narrow rim of cortex.

The metastases varied in size from a few millimeters to more than 2 cm. in diameter. The growth was of a grayish white or reddish gray color and usually rather translucent. The lesions were always soft, and while they were frequently hemorrhagic, they showed comparatively little tendency to mass necrosis.

The mode of growth was peculiar in that it produced a comparatively uniform expansion of the gland with gradual obliteration of both cortex and medulla but showed little or no tendency to invade the surrounding tissues. On the other hand as long as the growth was confined to the substance of the gland, there was practically no reaction of any kind about the tumor cells and none in the surrounding tissues. When the capsule became involved, however, there was a slight



TEXT-FIG. 6. Relative incidence of heart metastases.

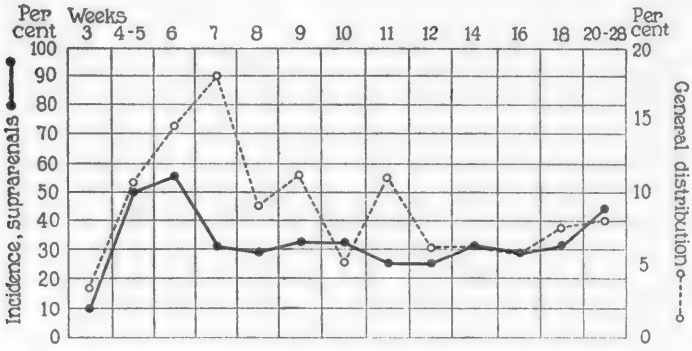
granulomatous reaction but still no encapsulation of the tumor mass such as occurred at times in all other organs with the exception of the hypophysis. In like manner, no cases of regressing or healed metastases were found.

The circumstances associated with the occurrence of suprarenal metastases were also unusual. In the first place, they developed early in the course of the disease, the period of maximum incidence being fully a week in advance of that shown by even such organs as the lungs, while a second peculiarity was the maintenance of a practically constant and comparatively high level of incidence from the 7th week onward (Text-fig. 7). Translating these facts into clinical terms, it may be said that suprarenal metastases occurred chiefly in two groups of animals: first, in cases of fulminating malignancy, and second, in animals that showed few or no lesions elsewhere. Some of these had definitely recovered from a more extensive involvement, while others gave no evidence of preexisting metastases in other organs. In brief, when both suprarenals were involved, death occurred,

but when only one was involved, there was no serious impairment of vitality on this account, and since suprarenal metastases did not undergo spontaneous resolution, a comparatively high level of incidence was maintained throughout the period of observation. The condition presented by many of these animals was that of an apparent immunity in the presence of an actively growing metastasis in one suprarenal.

Nowhere else was there an exact parallel of the conditions described and they are worthy of especial emphasis on account of their bearing upon phenomena of tumor growth and upon animal resistance.

Eyes.—Eye metastases may be considered to advantage in connection with the suprarenals since they presented some of the same peculiarities that were noted with metastases in those organs and possessed the additional advantage of permitting a correlation between clinical and pathological observations which is helpful in their interpretation.



TEXT-FIG. 7. Relative incidence of suprarenal metastases.

Metastatic growths were noted in the eyes of fourteen animals during life, and a few additional lesions were encountered at autopsy. The right eye alone was involved twelve times, the left twice, while in four animals, metastases developed in both eyes. In one or two instances, the growth was located in the choroid, but, as a rule, it arose from the iris or ciliary body and with few exceptions extended forward through the iris into the anterior chamber.

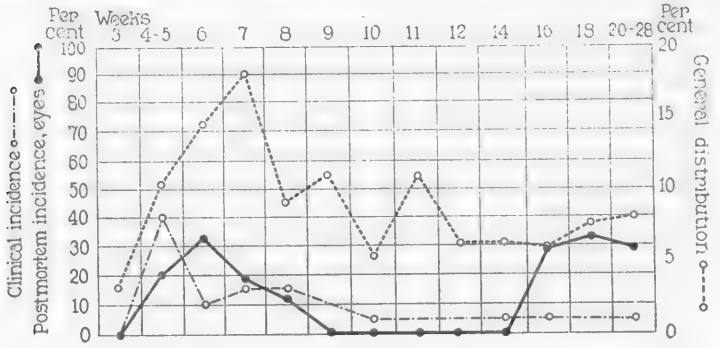
The presence of these metastases was first indicated by a focal or complete pericorneal injection or by the occurrence of hemorrhage into the anterior chamber associated with an irregular prominence in some part of the iris, usually towards the upper or outer margin. The growth of these tumors was very rapid and they presented the same general appearance as primary tumors in this location.

There was one peculiarity of these lesions which may be referred to briefly. In most instances, the cornea remained clear or showed only a slight clouding at the

outer margins, and this usually occurred very early and without reference to the extent of the growth. In exceptional instances, however, the entire cornea became very much clouded with an apparent roughening and dryness of the surface which reminded one of the condition described as xerophthalmia. Again, there was no constant relationship between the development of this condition and the extent of the tumor growth. Moreover, the change was not entirely permanent but cleared to some extent in animals in which the tumor regressed.

Eye metastases, as in the case of primary tumors, showed clearly defined periods of progression and regression followed by relapse. In some animals, the growth completely disappeared, leaving only a small fibrous scar, while in others the tumor remained active with no tendency to heal during a period of 3 to 4 months.

As in the case of the suprarenals, eye metastases occurred in two very different classes of animals: first, in animals with highly malignant tumors, and second, in



TEXT-FIG. 8. Relative incidence of eye metastases. Clinical incidence as compared with postmortem.

animals with few or no secondary lesions in other parts of the body. These facts are represented in the curve showing the presence of eye lesions at different times as determined by postmortem examination (Text-fig. 8). The curve shows a rapid rise during the 4th to the 6th weeks with a more gradual decline and a second elevation of equal extent during the later periods of observation. This final rise is artificial and is due to the fact that a number of animals with eye metastases were held under observation in order to determine what the outcome in these cases might be. If the existence of these lesions had not been known, this group of animals would probably have been distributed over the periods from the 7th week onward giving a curve not unlike that of the suprarenal metastases.

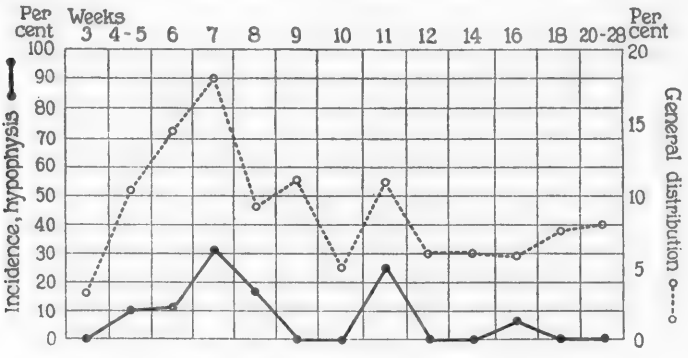
The curve of actual incidence (Text-fig. 8) assumes much this form. That is, if the time of clinical record be substituted for the record of postmortem examination, it will be seen that the majority of eye metastases occurred about 4 weeks

after inoculation and that there was a slight secondary increase during the 7th and 8th weeks. In brief, 80 per cent of the lesions occurred during the first 8 weeks.

This comparison of clinical and pathological data, when taken in conjunction with the history, is helpful in interpreting the results obtained in other organs where the curve of incidence indicates the operation of a similar group of factors.

Hypophysis.—Metastases in the hypophysis also presented a picture closely related to that of suprarenal metastases both as regards the character of the growth and the conditions under which they occurred.

As far as could be determined, the tumors arose from either the anterior or middle lobe and were identical in character with those in the suprarenals. They were usually small and, with one exception, were confined within the sella which showed a varying degree of enlargement, depending upon the size of the tumor. In one instance, the growth involved both the infundibular stalk and the entire area of the


Per cent
Weeks
3 4-5 6 7 8 9 10 11 12 14 16 18 20-28
Per cent
100 90 80 70 60 50 40 30 20 10 0
20 15 10 5 0
Incidence, hypophysis
General distribution o----o

TEXT-FIG. 9. Relative incidence of metastases in the hypophysis.

tuber cinereum, forming a mass in the base of the brain which was more than a centimeter in diameter.

As in the case of suprarenal metastases, there was practically no reaction about the growth in the hypophysis and there was no evidence that these lesions underwent spontaneous resolution.

There were two features of interest in the occurrence of hypophyseal tumors. The curve of incidence (Text-fig. 9) brings out in typical form the three waves of diminishing frequency which correspond with similar waves in the curves of many other organs. In fact, this group of lesions was confined almost exclusively to animals with widespread metastases. The exceptions occurred in animals with combined suprarenal and hypophyseal involvement in which few metastases had developed elsewhere or in which preexisting lesions in other organs had healed. In this respect, there was close agreement between the conditions under which metastases occurred in the suprarenals and hypophysis, indicating comparable

resistance to tumor growth. There were wide differences, however, in the curves of incidence which may be attributable on the one hand to differences of accessibility to tumor cells and on the other hand, to the fact that hypophyseal involvement almost inevitably caused death.

In the present paper, a description is given of secondary growths arising from direct extension of the primary tumor and from the implantation of cells on serous surfaces, together with metastases in the lungs, the liver, the kidneys, the heart, the suprarenals, the eyes, and the hypophysis. Metastases in other organs and tissues will be described in Part 2 of this paper.

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2. Pearce, L., and Brown, W. H., *J. Exp. Med.*, 1923, xxxvii, 811.

EXPLANATION OF PLATES.

The illustrations are reproductions of photographs which have not been retouched. Statements of time are from the date of inoculation.

PLATE 25.

FIG. 1. 52 days. Physical condition of animal fair; beginning weakness and slight loss of weight; loss of bladder control. Killed. The most striking features of the condition presented by this animal were the extension of the growth upward in the retroperitoneal tissues to the base of the neck and the formation of a profusion of implantation metastases some of which were extremely large and actively growing with little or no necrosis anywhere. The serous cavities were all filled with a blood-stained fluid. There were comparatively few metastases outside the abdominal and thoracic cavities. Note the well nourished condition of the animal, which may be contrasted with Fig. 3. × ½.

PLATE 26.

FIG. 2. 49 days. Extreme weakness; moderate loss of weight. Killed. This photograph shows a comparatively small primary tumor in the right testicle which has invaded the tunics and extended along the retroperitoneal lymphatics to the lumbar and perirenal regions. There is a small metastatic nodule in the left testicle, and there are a few lesions in the liver, but the growth is most marked in the kidneys. Many of the lesions show a hemorrhagic tendency, and there is a diffuse subcapsular hemorrhage in the left kidney. There were numerous mestastases in other parts of the body. Natural size.

PLATE 27.

FIG. 3. 49 days. Extreme weakness and emaciation. Killed. This animal is intended primarily to illustrate the condition present in fulminating malignancy, although the progress of the disease was not so rapid as is sometimes seen. There were metastases in all the abdominal and thoracic viscera, including the spleen, and in the cerebral meninges, the hypophysis, the eyes, and widespread lesions in the skin and subcutaneous tissues, the deep and superficial lymph nodes, the muscles, and the bones. Many of these metastases are marked by arrows to facilitate identification. This photograph should be examined in connection with Fig. 4 which shows the lesions over the back of the same animal. The lesions of especial interest which may be seen are those in the heart, the skin and subcutaneous tissues, superficial lymph nodes, the muscles (thigh and jaw), the bones of the jaw, and the extensive hemorrhage in the right kidney. The animal also shows a wasting of the fat and of the muscles, especially noticeable in the lumbar region. About $\frac{1}{3}$.

PLATE 28.

FIG. 4. The same animal as in Fig. 3. Note the number and size of the cutaneous and subcutaneous metastases and the prominence of the right shoulder, which was due to a growth in the muscle which possibly arose from the scapula. Natural size.

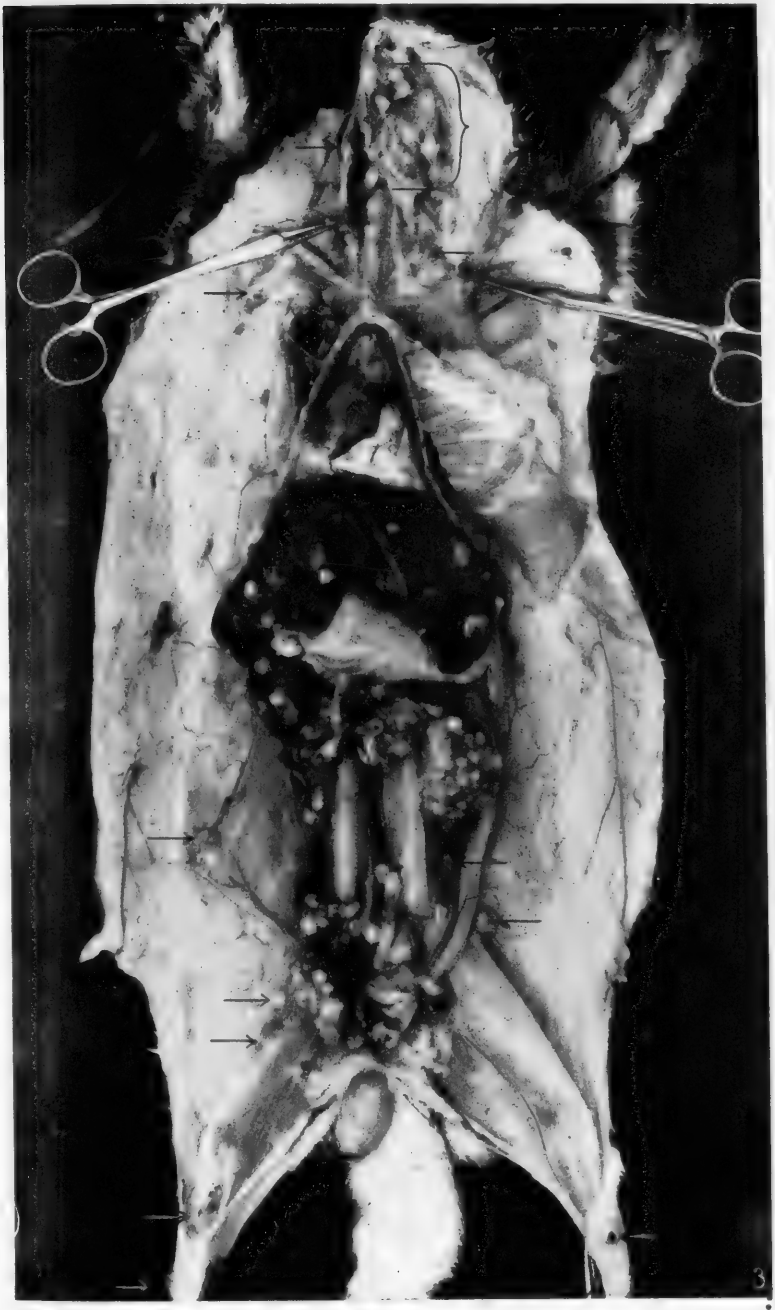
PLATE 29.

FIGS. 5 and 6. The animal whose liver is shown in Fig. 5 died 82 days after inoculation; that from which the liver in Fig. 6 was taken showed an extremely rapid development of skin and bone metastases during the 4th week after inoculation with progressive weakness and emaciation and was killed on the 29th day. Natural size.

The metastases in these two livers illustrate a difference in the character of the lesions seen in cases of rather slowly progressive malignancy and in those that are truly fulminating. The difference in the lesions and the associated change in the organ are typical of the conditions presented by all other organs under similar circumstances and show to what extent the number and character of the lesions present can be taken as an index of malignancy or more properly of animal resistance, since varying degrees of the condition illustrated may be seen in different animals of the same series.

(Pearce and Brown: Malignant tumor of the rabbit. V.)

(Pearce and Brown: Malignant tumor of the rabbit. V.)



(Pearce and Brown: Malignant tumor of the rabbit. V.)

(Pearce and Brown: Malignant tumor of the rabbit. V.)

(Pearce and Brown: Malignant tumor of the rabbit. V.)

STUDIES BASED ON A MALIGNANT TUMOR OF THE RABBIT.

V. Metastases.

Part 2. Description of the Lesions with Especial Reference to Their Occurrence and Distribution.

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(Received for publication, May 25, 1923.)

This paper continues the description of metastatic growths contained in Part 1. Consideration of this subject may be resumed with the lesions of the skin and subcutaneous tissues.

Skin and Subcutaneous Tissues.—Comparatively few animals showed metastases in the skin or subcutaneous tissues, but in view of the difficulty experienced in obtaining a growth in these tissues by direct inoculation, the fact that metastases occurred at all is of the greatest significance. With one exception, the lesions were confined to the trunk, the neck, and the proximal segments of the legs.[1] They were most numerous over the anterior part of the body, especially over the back and in the region of the shoulder girdle, and in general followed the lines of blood vessels. In one instance, there were solitary lesions about 1 cm. in diameter symmetrically located on the shins.[2]

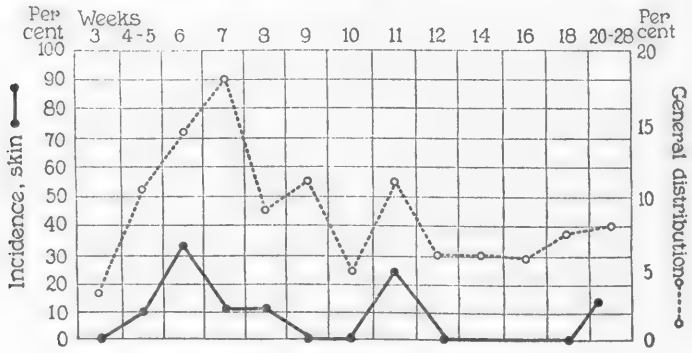
As a rule, cutaneous metastases were small, shotty nodules of an opaque white or translucent appearance, although some of them were of a purplish red color. Occasionally, the lesions measured as much as 1 cm. in diameter, and the larger ones were usually soft and hemorrhagic in character.

The curve of incidence for cutaneous metastases (Text-fig. 1) differed very little from that of the hypophysis with an actual incidence

[1] Part 1, Figs. 3 and 4 (the same animal).

[2] Part 1, Fig. 3.

of metastases comparable to that of the skin (11.4 per cent). The majority of the lesions occurred somewhat earlier. In fact, cutaneous metastases were, as a rule, associated with cases of fulminating malignancy.[1] There was only one instance in which a growth in the skin underwent spontaneous resolution, and there were two animals from early generations in which the disease pursued a chronic but fatal course. Otherwise, the extent of the cutaneous involvement was directly proportional to the severity of the disease and constituted the most reliable clinical index of malignancy. This might have been expected from the structure of the skin and from its known resistance to direct inoculation of the tumor cells.



TEXT-FIG. 1. Relative incidence of skin metastases.

From the data available, it appears that metastases occurred in the subcutaneous tissues under much the same conditions as in the skin, but on account of the possibility of confusing lesions of this class with metastases in subcutaneous lymph nodes, no attempt has been made to analyze them in detail.

Finally, the development of metastases in the skin and subcutaneous tissues furnishes what is perhaps the most conclusive evidence of the occurrence of marked alteration in animal resistance induced by the growth of the tumor itself.

Muscles.—Metastases occurred in the skeletal muscles with slightly greater frequency than in the skin. They were widely distributed but showed a distinct predilection for the muscles of the thigh, the face,

the thorax and shoulder girdle, and the abdominal walls. Other muscles involved were those of the lumbar region, the neck, and the anterior extremities in the order of frequency given. Metastases occurred also in the diaphragm, but in most instances it was impossible to distinguish between implantations and lesions which developed within the muscle itself.

Muscle metastases were usually few in number but were inclined to be rather large (1 to 2 cm. in diameter), soft, and hemorrhagic.[2] They were usually associated with a well marked cellular reaction, but there was little tendency to encapsulation, and as far as is known, they did not undergo spontaneous healing.

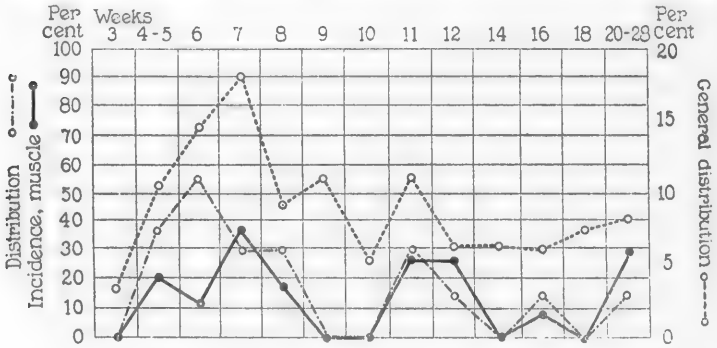
In considering the incidence of muscle metastases with reference to time and the conditions under which they occurred, the factor of distribution, or the number of foci affected, has to be taken into account. As long as one is dealing with single or paired organs, distribution can be disregarded, but in the case of such organs as the muscles, the bones, and the lymph nodes, the number of foci affected becomes a matter of considerable importance.

Muscle metastases were confined to animals with relatively malignant tumors, but occurred with almost equal frequency at three widely separated periods, as shown in Text-fig. 2. That is, from the standpoint of animal incidence, lesions of this class were almost equally divided between slowly progressive and rapidly progressive cases of malignancy. If the curve of muscle metastasis were plotted upon this basis, however, it would be somewhat misleading in that the most extensive muscle involvement was encountered among animals with the most malignant tumors, while there were comparatively few lesions in those with slowly progressive tumors. These facts are brought out by correcting the curve of animal incidence according to the number of foci involved, which places the correct emphasis upon the factor of malignancy and at the same time brings the curve in harmony with those of other metastases whose incidence was determined by a similar group of factors.

Bones.—Bone metastases occurred with about the same frequency as metastases in the muscles or the thymus. In fifteen animals, the lesions were distributed as follows:

Facial bones	12
Calvaria	6
Thorax	4
Posterior extremities	4
Spinal column	3
Anterior extremities	1

There were doubtless other metastases which were overlooked, since the bones could not always be examined with minute care. This list is sufficient, however, to indicate the general tendencies of distribution, the important features of which were the great preponderance of lesions in the axial skeleton and in bones with a comparatively direct blood supply. Moreover, there was a decided preference for cancellous


Per cent
Weeks
3 4-5 6 7 8 9 10 11 12 14 16 18 20-28
Per cent
100 90 80 70 60 50 40 30 20 10 0
20 15 10 5 0
Distribution
Incidence, muscle
General distribution

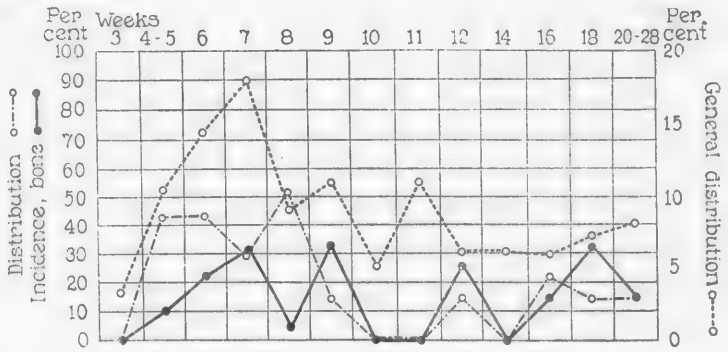
TEXT-FIG. 2. Relative incidence and distribution of muscle metastases.

bone, and even in the case of the long bones of the extremities, the growth was more abundant in the epiphyses than in the shaft.

There were a few instances in which metastases appeared to have developed beneath the periosteum in such locations as the mandibles,[2] the distal ends of the ulna, and the external malleoli (not included in the above classification). As a rule, however, the growth arose within the bone, but not infrequently extended outward with the formation of prominent swellings over the surface, and in several instances large fungating masses developed about the teeth. The tumor also showed a tendency to localize in or to extend to the cranial sinuses, and there were two instances in which metastatic growths filled the middle ear on one or both sides.

The bone metastases that were found were rather large, extremely soft, and inclined to be hemorrhagic in character. They produced comparatively little bone destruction, which was probably due to rapid and invasive growth combined with short duration. There was a distinct reduction of bony tissue, however, and spontaneous fractures occurred in a few animals.

No apparent reaction occurred about the tumor mass within the bone, and there was comparatively little about that portion of the growth which extended into the surrounding tissues. In like manner, as far as is known, no bone metastases underwent spontaneous absorption.


Per cent
Weeks
3 4-5 6 7 8 9 10 11 12 14 16 18 20-28
Per cent
100 90 80 70 60 50 40 30 20 10 0
20 15 10 5 0
Distribution
Incidence, bone
General distribution

TEXT-FIG. 3. Relative incidence and distribution of bone metastases.

From the standpoint of animal incidence, this group of lesions was again almost equally divided between cases of fulminating malignancy and animals with relatively malignant but slowly progressive tumors. The extent of the involvement, however, was much greater in the first group of animals, as shown in Text-fig. 3. In these respects, there was a close analogy between bone and muscle metastases, and there are points of resemblance between the two curves, but they do not coincide. The chief difference appears to lie in a more uniform distribution of bone metastases among four groups of animals, with a slightly larger proportion of cases occurring during the later periods of observation. In other words, there is evidence of a further shift of incidence from the first to the second half of the scale, while the relative extent of

involvement occurring at the two periods is still greatest in animals with rapidly progressive tumors.

Lymph Nodes.—On account of the wide distribution of small masses of lymphoid tissue and the difficulty in determining the exact point of origin of metastases in such places as the subcutaneous tissues, the retroperitoneal tissues, and the mediastinum, it was impossible to observe the same degree of accuracy in recording the occurrence of metastases in lymph nodes that was possible elsewhere. Hence, in order to avoid placing undue emphasis upon this group of metastases, only those lesions were included which could be definitely localized in lymph nodes. When such a distinction could not be made, metastases were recorded as occurring in the tissues of the region concerned. In this way, the actual number of lymph node metastases was undoubtedly underestimated, and this caused a corresponding error of increase in the number of metastases referred to subcutaneous, retroperitoneal, and mediastinal tissues and to the thymus. Nevertheless, lymph node metastases ranked high in point of incidence.

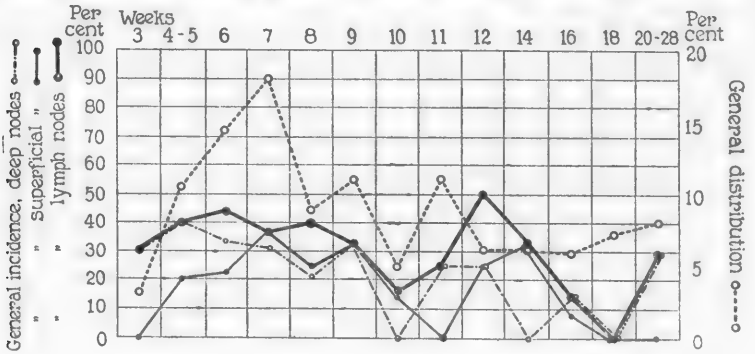
The distribution and order of frequency of involvement of different groups of nodes were as follows:

Retroperitoneal	18
Mesenteric	10
Posterior cervical	8
Axillary	7
Inguinal	7
Mammary	6
Submaxillary	5
Peritracheal	5
Peribronchial	4
Posterior axillary	3
Anterior cervical	3
Flank	1
Auricular	1

The only groups of nodes of any considerable importance (size) which are not represented in this list are the main mesenteric mass and the nodes immediately connected with it, the deep cervicals, and the popliteals. This is of especial interest when it is recalled that these are the largest nodes in their respective regions. The absence of metastases from the popliteals is not so surprising, since to a very large

extent metastases were confined to the head, neck, and trunk, but they did occur in the skin, muscles, and bones of the posterior extremities and even in the loose areolar tissue of the popliteal space. It is more difficult to account for the absence of metastases from the mesenteric nodes and from the deep cervicals, except upon the basis of some structural or functional peculiarity, since all the neighboring nodes and tissues were affected with comparative frequency.

Lymph node metastases were usually rather large[2] and of a grayish pink color. They were soft and frequently hemorrhagic but showed comparatively little necrosis, except in the case of retroperitoneal and mediastinal nodes. These nodes frequently contained


Per cent
Weeks
3 4-5 6 7 8 9 10 11 12 14 16 18 20-28
Per cent
100 90 80 70 60 50 40 30 20 10 0
20 15 10 5 0
General incidence, deep nodes
" superficial "
" " lymph nodes
General distribution

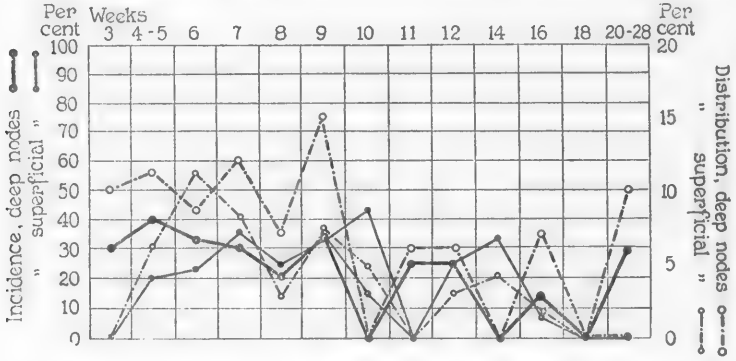
TEXT-FIG. 4. Relative incidence of lymph node metastases as compared with the incidence of involvement of superficial and of deep nodes.

large necrotic masses surrounded by a thick fibrous capsule, and it appeared that healing of metastases in the retroperitoneal lymphatics was not infrequent and that it probably occurred in the mediastinal nodes also. In these respects, there was a decided difference between the deep and superficial lymph nodes. In such nodes as the superficial cervicals, the axillaries, etc., there was practically no tissue reaction about the tumor cells, and no regressive changes occurred, except in one animal with a metastasis in the right inguinal node.

The curve of incidence for lymph node metastases was quite different from that of any group of lesions thus far considered. If all classes of nodes are included, as in Text-fig. 4, there is a high and relatively

constant level of incidence from the 3rd to the 9th weeks after inoculation with a second period of increased frequency of even greater extent occurring late in the course of the disease.

If this curve is divided on the basis of deep and superficial nodes (Text-fig. 4), a different picture is presented in that while both groups of nodes show a comparatively high incidence during the earlier periods of observation, those in the drainage area (deep nodes) of the primary tumor show a sustained elevation during the earlier weeks followed by a gradual decline, while the superficial nodes are uninvolved at the beginning but show a gradual rise with some fluctuations and a sharp drop at the 10th week, followed by a second period of



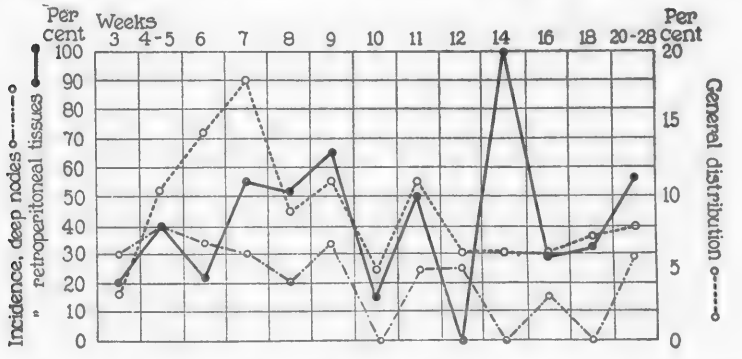
TEXT-FIG. 5. Comparison of the relative incidence and distribution of metastases in deep and in superficial lymph nodes.

increased frequency. This curve is not unlike that of bone and muscle metastases but still shows certain distinct differences.

If the curves for deep and superficial nodes are further modified upon the basis of the extent of involvement, or the number of nodes affected, the differences between the two groups of nodes become still greater, as shown in Text-fig. 5. Under these circumstances, the superficial nodes give a curve much like that of the lungs or the liver, showing a maximum involvement, which in this curve really means incidence, at the 6th week with a reduction during later periods, while the maximum involvement of the deep nodes occurs much later and is decidedly more marked during late stages of the disease.

The deep nodes of the abdominal and thoracic groups were involved in all classes of animals without especial reference to the severity of the disease, but in as far as incidence alone is concerned, the involvement was greater in slowly progressive cases than in fulminating cases of malignancy, while with the superficial nodes this condition was reversed. The significance of these relationships will be more apparent when we have considered the incidence of metastases in such locations as the retroperitoneal tissues and the mediastinum.

Retroperitoneal Tissues.—As has been intimated, it was impossible to draw a sharp line of distinction between metastases which were distributed along the course of lymphatics in the retroperitoneal


Per cent
Weeks
3 4-5 6 7 8 9 10 11 12 14 16 18 20-28
Per cent
100 90 80 70 60 50 40 30 20 10 0
20 15 10 5 0
Incidence, deep nodes
" retroperitoneal tissues
General distribution

TEXT-FIG. 6. Comparison of the relative incidence of metastatic growths in the deep lymph nodes and the retroperitoneal tissues.

tissues and those which were localized in well defined masses of lymphoid tissue. In describing this group of lesions, therefore, the main object is to bring out certain points of similarity and of difference between the two groups of metastases.

The lesions were alike in all respects and they occurred under much the same circumstances. If the curves of incidence are compared, however (Text-fig. 6), it will be seen that during the first 6 weeks, lymph node metastases were given a higher rating than those in retroperitoneal tissues. This indicates that the earlier lesions, being relatively small and not especially numerous, were more accurately localized than at other times. At the 12th week the same relations

obtained and may be attributed to the same causes. On the whole, it appears that the two curves tend to supplement each other. From point to point their direction is usually the same, but occasionally they assume opposite directions, as at the 6th, the 11th, the 12th, and the 14th weeks. Moreover, the curve of lymph node metastases gradually drops to a lower and lower level, while that of retroperitoneal metastases assumes a relatively greater importance in chronic or slowly progressive forms of disease. The significance of the two groups of lesions appears, therefore, to be essentially the same, the difference between them being largely one of degree.

A further relationship to be noted is that between the general distribution of metastatic growths, or the severity of disease, and the occurrence of this group of lesions. By comparing these two curves (Text-fig. 6), it will be seen that there is a decided lack of agreement, which suggests that the conditions which determine the occurrence of retroperitoneal metastases and metastases in general are not the same and that the conditions which favor the development of retroperitoneal metastases are of relatively greater importance during the late than during the early stages of the disease or are of greater importance in cases of relatively benign tumor growth than in those of fulminating malignancy.

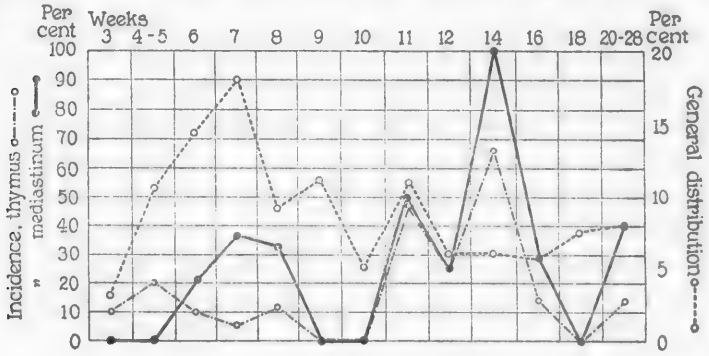
Mediastinal Tissues.—Metastases in the mediastinum presented much the same difficulties of classification as retroperitoneal metastases. In some animals, the growth in the retroperitoneal tissues extended upward into the mediastinum with the formation of tumor nodules along the course of the lymphatics, in the lymph nodes and areolar tissue of the mediastinum, and in the thymus as well.[3] In other instances, the lesions present appeared to be independent of any growth in the retroperitoneal tissues.

Exclusive of the thymic mass, mediastinal metastases occurred in twenty-nine animals, or in 25.4 per cent of those showing metastases. The lesions were usually few in number but rather large, varying from about 0.5 to 2.0 or 3.0 cm. in diameter, and were either attached to some neighboring structure or were freely movable in the loose areolar tissues.

[3] Part 1, Figs. 1 and 3.

The character of the growth also varied in individual animals depending upon the progress of the disease. In instances in which the disease was of a rapidly progressive or fulminating character, the growth was composed of a soft, grayish pink mass with a delicate covering of connective tissue, while in animals in which the disease pursued a more chronic course, the lesions were larger and inclined to be necrotic and were usually enclosed in a dense fibrous capsule.

As has been intimated, mediastinal metastases occurred under two entirely different sets of conditions. They were of frequent occurrence in cases of high malignancy but were even more frequent in animals with slowly progressive tumors and with comparatively few metastases


Per cent
Weeks
3 4-5 6 7 8 9 10 11 12 14 16 18 20-28
Per cent
100 90 80 70 60 50 40 30 20 10 0
20 15 10 5 0
Incidence, thymus mediastinum
General distribution

TEXT-FIG. 7. Comparison of the relative incidence of secondary growths in the mediastinum and in the thymus.

elsewhere except in the retroperitoneal tissues. Represented graphically (Text-fig. 7), there were two periods during which mediastinal metastases occurred, one early and the other late, while the curve as a whole shows an upward rather than a downward trend from the 4th to the 14th weeks.

The curves for the two periods, however, are so different as to suggest that lesions represented by the two divisions may be referable to different causes. Moreover, the first part of the curve closely resembles corresponding parts of a number of the curves previously considered, while the second half is almost a duplication of the corresponding section of the curve of retroperitoneal metastases. The significance of this section is, therefore, quite obvious.

Thymus.—Metastases occurred in the thymic mass with about the same frequency as in the bones and the muscles, but it is uncertain whether any of these lesions could be regarded as having arisen within the substance of the thymus itself. Some of them were definitely located in small masses of lymphoid tissue included within the capsule of the thymus. These lesions were analogous in all respects to metastases in the superficial lymph nodes and occurred under exactly the same conditions; that is, in cases of fulminating malignancy.

In other animals, the thymus was replaced by a tumor mass which virtually filled the superior mediastinum and frequently gave rise to marked respiratory distress. These tumors were surrounded by a dense fibrous capsule and usually showed extensive necrosis but comparatively little absorption of the necrotic material; that is, there was no definite evidence of healing.

In only one instance were metastases found in a thymus that appeared to be normal. As in the case of the mediastinum, thymic metastases occurred in two types of disease, the one very acute and the other chronic, but in both instances, there was extreme atrophy of the thymus. Metastases were relatively infrequent among the animals killed within the first 10 weeks after inoculation (Text-fig. 7), while during the later stages of the disease they were almost as frequent as mediastinal metastases, and the curves of incidence for this period virtually coincide.

Perirenal Fat.—Scattered nodules of tumor growth occurred in the perirenal and lumbar fat of a considerable number of animals, and occasionally there was a striking tendency to localization of metastases in the lumbar fat in general, but especially in the perirenal tissues. The conditions under which this occurred are not entirely clear, but the feature of interest was the accumulation of large masses of tumor tissue in the form of multiple nodules which at times completely surrounded the kidneys while the kidneys themselves were unaffected. These animals showed few implantations and few metastases in other organs or tissues.

It appeared that the growth was the result of a rapid distribution of tumor cells by way of the lymphatics, and if this assumption is correct, the absence of renal involvement, or the involvement of other organs, is of especial significance.

Organs and Tissues in Which Metastases Were Infrequent.

In the preceding pages, a description has been given of metastases and the conditions under which they occurred in those organs and tissues which were most often affected. In contrast with these, there were several groups of organs which are of interest on account of the fact that they were rarely or never involved by metastatic growths. These include the gastrointestinal tract, the pancreas, the genitourinary organs, the central nervous system, the spleen, the thyroid and the parathyroids, and the pineal gland.

Gastrointestinal Tract.—There was only one instance of a clearly defined metastasis in the walls of the stomach, and even implantations on the serous surface were comparatively rare. In the one instance recorded, the growth presented the typical appearance of a peptic ulcer. The lesion was about 1 cm. in diameter with a depressed ulcer at the center surrounded by an elevated margin which was composed of tumor cells located chiefly in the submucosa.

The intestine was affected more often and was among the most frequent locations of implantation metastases. As a rule, however, the lesions in the intestine were small and were located either in the subserous or submucous tissues. In two instances, comparatively large tumors developed, and one of these led to an intussusception which caused the death of the animal.

Pancreas.—In view of the frequency of metastases in the omentum and mesentery (implantation), it is remarkable that there were only five instances in which secondary growths occurred in the pancreas or in that portion of the mesentery occupied by the pancreas.

Genitourinary Organs.—Omitting the kidneys, the genitourinary organs were rarely involved. There were comparatively few implantations on the bladder and only one metastasis of any considerable size in the bladder walls. This was situated in the trigone and formed a nodule about 8 mm. in diameter which projected from the inner surface. The prostate and seminal vesicles were affected only once by a growth which extended downward from the retroperitoneal tissues.

In like manner, metastases to the uninoculated testicle occurred in very few animals, and even extensions and implantations on the

cord and tunics of the uninoculated testicle were comparatively rare. In view of the ease with which successful inoculations were obtained in the testicle, the low incidence of metastatic growths in the uninoculated testicle is especially significant as an indication of the relative importance of so called suitability of soil and of other factors in determining the location of secondary growths.

Central Nervous System.—There were five instances in which metastases were found in the meninges of the brain or cord. The origin of these tumors was not certain in all cases. Those in the cranial cavity appeared to have developed in the meninges, while those in the spinal canal arose either in the bones or in the surrounding tissues and entered the canal through the intervertebral foramina.

It is a striking fact that not a single case of brain or cord metastasis occurred. Here again we are confronted by the total absence of metastases from an organ which is highly susceptible to inoculation and in which primary tumors grow with the greatest ease.

Spleen.—Out of a total of 191 animals examined, there were four in which metastases occurred in the spleen as compared with thirty cases of metastasis to organs of a purely lymphoid character. The lesions were small and produced little or no local reaction, and all of them occurred in cases of fulminating malignancy. These findings are probably in accord with general experience, but they are nevertheless significant as an indication of the presence of conditions of some kind which prevent the occurrence of metastases in the spleen.

Thyroid and Parathyroids.—Metastases in the thyroid are of especial interest in comparison with the conditions presented by the suprarenals and the hypophysis as regards both the frequency of occurrence and the character and extent of the local reaction. There were thirty-seven animals with suprarenal metastases and thirteen with metastases in the hypophysis, while there were only two instances recorded in which visible metastases occurred in the external parathyroids and four in the thyroid. In two of these animals, it appeared, however, that the growth might have arisen from an attached or internal parathyroid rather than from the thyroid itself. There was one other animal with a metastasis in the trachea beneath the isthmus of the thyroid, but as far as could be determined, the thyroid itself was not involved.

These growths were comparatively small, the two largest measuring not more than 6 to 8 mm. in diameter, while the others were barely visible to the unaided eye. All of them occurred in animals with extremely malignant tumors, whereas suprarenal and hypophyseal metastases were of frequent occurrence in animals with slowly progressive tumors or with few metastases elsewhere.

The low incidence of gross metastases in the thyroid does not appear to be due to a failure of tumor cells to reach the thyroid. Numerous small collections of cells were found by microscopic examination, but in all instances, they were submerged beneath a massive lymphocytic and fibroblastic reaction which again was in striking contrast with the absence of such reactions in the suprarenals and hypophysis.

When it is recalled that gross metastases occurred in only a few animals with tumors of a most malignant character, indicative of an exceptionally low constitutional resistance, it would appear that the factors of constitutional and local resistance are of especial importance in determining the occurrence of thyroid metastases, and this probably applies to the parathyroids as well.

DISCUSSION.

The description which has been given of the metastases produced by this tumor will serve to convey a general conception of this feature of the disease. It will be seen that here again we are dealing with conditions which in many respects are closely analogous to those that obtain in man but with the added advantage of being able to produce an almost endless series of changes from a given tumor stock and of being able to interrupt the process at any point for the purpose of tracing the successive stages in its development. It is thus possible to link together cause and effect in a way that at best is extremely difficult from the study of human material.

Reviewing the facts presented, one is first impressed by the unusual possibilities of this tumor for the production of metastases, affecting, as they may, nearly all the organs or tissues of the body. Still, it will be seen that there is the greatest diversity in the results obtained in different animals under precisely the same experimental conditions. In the first place, a distinction may be drawn between animals

that develop metastases and those that do not, while a further division may be made between animals that apparently recover after metastases have developed and those in which the disease goes steadily onward.

Moreover, among the animals of the last group, the character and distribution of the lesions and the course of the disease vary to such an extent in different animals that, if the two extremes were compared, one could hardly recognize them as manifestations of the same etiologic agent. In one group of animals, metastases develop slowly and are confined largely to the region of the lymphatics which lead upward from the primary tumor to the mediastinum, while in the other, the development of metastases takes place with incredible rapidity and few organs or tissues are spared. In the one instance, the disease is slowly progressive, while in the other, it is fulminating in character. Between these two extremes, one finds various modifications and combinations of the two outstanding types of metastatic involvement.

Finally, by a similar process of analysis, it may be seen that practically the same conditions obtain with reference to the development of metastases in different organs of a given animal or in the homologous organs of different animals.

At first sight, all of these differences may appear to be largely matters of chance but upon closer examination, it will be seen that, in as far as individual organs and tissues are concerned, there is a remarkable consistency in their behavior and that such variations as occur in a given organ or tissue bear a definite relation to the variations which occur in other organs of the same animal. In brief, it will be seen that the peculiarities displayed by individual animals are founded upon constitutional differences which are most clearly expressed in the behavior of the individual elements which form the more complex animal organism.

If animal resistance is considered from this point of view, it is obvious that the most direct method of approach to the problem of resistance to tumor growth is by a careful investigation of conditions which obtain in individual organs and tissues and the relations which exist between one group of organs and another. From investigations of this kind, it may be possible to reconstruct the whole.

This conception is applicable to the study of metastases, since they represent an expression of one form of interaction between tumor and host, and the data bearing upon the subject of metastasis will be taken up from this point of view in the next paper of the series.

SUMMARY AND CONCLUSIONS.

As a part of a general investigation of a malignant tumor of the rabbit, the phenomena of metastasis were studied in a group of 191 animals. The results of this investigation are presented from the standpoint of the relation of metastasis to animal resistance. The incidence and distribution of different classes of lesions are given and the peculiarities of the growth in different organs and tissues are described with especial reference to the time and circumstances of their occurrence and the relation of metastases in one organ or tissue to those of another.

It was found that while the picture presented by different animals varied greatly, there was a remarkable degree of uniformity in the character of the lesions in a given organ or tissue and in the circumstances under which metastases occurred in a given location. It was thus possible to establish a relationship between the distribution of metastases and the function of animal resistance.

In brief, the conclusion was reached that the peculiarities of metastatic involvement displayed by individual animals were not entirely attributable to chance distribution of tumor cells but that they were founded very largely upon constitutional differences and that the picture presented in any given instance represented an expression of the interaction between tumor and host, the character and force of which were indicated by the nature and distribution of the lesions or by the organs and tissues affected.

STUDIES BASED ON A MALIGNANT TUMOR OF THE RABBIT.

V. Metastases.

Part 3. Factors That Influence Occurrence and Distribution.

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(Received for publication, May 25, 1923.)

In studying phenomena of metastasis of spontaneous tumors, whether in laboratory animals or in man, one has but a single example of the results of interaction between tumor and host, and while it is possible to accumulate large groups of cases composed of tumors of similar or even identical character, one is still confronted by the fact of individuality or a lack of knowledge of how a given tumor might behave in another individual. Our knowledge of phenomena of metastasis is founded largely upon the study of such material, but in spite of the difficulties referred to, it has been possible to show that tumors of a given class tend to metastasize by certain paths and to localize in certain parts of the body. It has also been found that, while the great majority of tumors of a given type show a remarkable degree of conformity in their general behavior, there are striking exceptions to these rules, and that a wide range of possibilities is presented by almost any variety of malignant tumor. Moreover, it is well known that tumors ordinarily regarded as benign may upon occasion assume malignant characteristics and give rise to metastases in distant organs.

In brief, our experience with spontaneous tumors has led to the creation of a conception of essential conformity in the behavior of tumors of a given class and of possible variations which is of inestimable value in dealing with malignant tumors. At the same time, comparatively little is known of the underlying causes for this behavior. No one knows whether these features of tumor growth are attributable to some tendency on the part of the tumor cell or whether after all the conformity in behavior may not be referable to the same causes as are responsible for the development of the tumor. At any rate, the objective evidence of cellular activity is the thing that has been most forcibly impressed upon us by our contact with malignant disease, and it may be that this experience has tended to magnify our conception of the relative importance of the tumor cell as an aggressive agent with selective capacities and to minimize the impor-

tance of other factors. In the same way, we have been led to believe that tumors are exempt from the general laws or principles governing pathological action or that they are autonomous and obey no known laws.

As long as our knowledge is confined to facts that may be gained from a study of spontaneous tumors and the single expression of action of a given tumor, it is difficult to escape such conclusions, but our point of view may be greatly enlarged by multiplying instances of the action of a given tumor stock and by varying the conditions of its activity. If, therefore, nothing more could be gained from the study of transplanted tumors, it would still be worth the while to examine into the one problem of the conformity of tumors to the action of principles of any kind with a view to determining the causes which are responsible for the uniformity in the behavior of tumors of a given class and the peculiarities which characterize the behavior of different classes of tumors. In other words, to what extent are the characteristics of tumor growth due to essential attributes of the tumor cells, and how far may these cells be influenced by factors which are within the host or by factors entirely distinct from either but operative through the host rather than directly upon the tumor cells? This is the essence of the problem of metastasis.

In the present instance of a rabbit tumor, the opportunity was afforded of making a careful study of the animal with the spontaneous tumor (1) and since then a long series of observations has been carried out on tumors derived from this parent stock (2–4). The spontaneous tumor metastasized both by way of the lymphatics and the blood stream, the organs affected being the regional lymph nodes, the lungs, the liver, the spleen, the bones, and the kidneys, but from a study of the spontaneous tumor alone, it would be impossible to say what the conditions were that determined this particular distribution of metastases. The picture presented might be regarded as a matter of chance or as an indication of a predilection of the cells of this tumor for growth in certain organs or as a condition determined by the mode of cell distribution. On the other hand, it might be viewed as an indication of resistance of different organs or tissues to the growth of the tumor cells or as a combination of all of these factors and perhaps of others. At any rate, these are among the possibilities that have to be considered, and the results obtained from the study of transplanted tumors may be analyzed with a view to determining how far the influence of such factors can be traced or the extent to which they have dominated the behavior of the tumor.

The incidence and distribution of metastases, the characteristics of the growth in different locations, and the circumstances of their occurrence, as well as the fate of lesions in different organs were described in the preceding papers of this series (5, 6) and will be used here as the basis for a discussion of factors that have influenced the occurrence and distribution of metastases.

DISCUSSION.

As regards the rôle of the tumor cell, it may be said that the picture presented by the original animal has never been duplicated. During the first twenty generations, no distant metastases were obtained in normal animals inoculated either in the skin or the subcutaneous tissues, and, although a great variety of lesions was produced by intratesticular inoculation, in no instance was there such a preponderance of metastases in the liver, spleen, and bones. From this, it is at once apparent that the tumor *per se* was not the sole cause for the picture presented by this animal but that there were other factors peculiar to the animal itself that played a part in determining the result.

In like manner, it must be apparent that, in as far as any inherent quality of the tumor cell itself is concerned, all animals and all organs or tissues may be regarded as being upon an equal footing. Still, it was found that even animals of the same series might show the greatest diversity in results so that, while the tumor cell must be considered as an essential element in the equation, it is obvious that its activities were limited by outside influences which were capable of producing an almost endless series of variations even though all experimental conditions were virtually constant, except those that concerned the individuality of the host.

The problem of metastasis may then be narrowed down to two main groups of factors; namely, accessibility of the part to tumor cells in a viable condition and the ability of the cells to grow in a particular organ or tissue or, so to speak, phenomena of cell transport and of cell nutrition in its broadest sense. The key to the solution of the problem lies, therefore, in conditions that determine distribution of cells, conditions that affect the viability of the cells during transport, and the ability of the cell to grow where it becomes lodged.

As has been pointed out, the mode of growth of this tumor is such as to render it practically certain that some dissemination of cells occurs early and in all animals with actively growing tumors and that cells are distributed to distant parts of the body by two separate and distinct paths, the blood stream and the lymphatics. However, mere entrance of tumor cells into the blood stream or lymph channels is not sufficient to insure the development of metastases. In fact, growth was rarely obtained by intravenous inoculation of normal animals, and there was virtually a complete failure of the cells to grow in distant organs of upwards of 40 per cent of the animals inoculated in the testicle, although it is known that, in all such animals, there were tissues in which the cells might have grown with ease provided they could have reached such places in a viable condition. Under ordinary circumstances, therefore, it is obvious that many cells entering the blood stream or the lymphatics are disposed of in such a way as to prevent their growth, and apparently this mechanism is more efficient in some animals than in others.

On the other hand, it has been seen that, when metastases do occur, a certain degree of order and of system prevails as regards the time and circumstances of

their occurrence, the distribution of the lesions, the character of the growth in a given location, and the reaction displayed by the surrounding tissues. Such a course of events is not altogether a matter of chance but is an expression of the action of conditions which have some part in shaping the course of the disease.

The nature of the factors concerned in these processes may be best appreciated by considering first those animals in which metastases occur as distinguished from those in which no metastases develop. If we may again make use of the course of disease in different groups of animals as an index of general resistance, it would be safe to assume that those animals in which the disease pursues a fulminating course are the ones that are most indifferent to the tumor and these were the animals that showed the most widespread distribution of metastases. They accounted for the wave of maximum incidence and of maximum distribution that occurred at about the 6th to the 7th weeks after inoculation.[1]

If one considers the incidence and distribution of metastases in all classes of animals during the first 6 to 8 weeks after inoculation, it will be noted that the peak of metastatic involvement was reached in practically all those organs and tissues which are not directly in the path of the lymphatics leading upward from the primary tumor. During the first 4 or 5 weeks, the lungs showed the highest incidence of metastases, followed by the suprarenals, the kidneys, the liver, and the heart, in the order given, with such organs as the eyes, the muscles, the superficial lymph nodes, the bones, the hypophysis, and the skin forming a second group in which the order of frequency was less definite.

The sequence in development of metastases in this group of organs suggests a distribution of cells by the blood and the order of incidence conforms fairly closely to that usually given for the lodgment of blood emboli, with a notable absence of metastases in such organs as the spleen and the central nervous system. (The area supplied by the mesenteric arteries is here omitted on account of the possibility of confusing blood- or lymph-borne metastases with implantations.) There is not an absolute agreement, however, but the evidence is sufficient to show that under favorable conditions, cells entering the blood stream may pass the pulmonary capillaries and be distributed to various parts of the body in a viable condition and that, in such cases, the location of metastases may be determined largely in accordance with principles of embolic distribution.

[1] Part 1, Text-fig. 2.

It is obvious, however, that mere mechanical distribution cannot determine growth. From what has been learned of the ability of the tumor to grow in different tissues by the use of direct inoculation and by the study of metastases, it is obvious that the possibilities of growth in any location are contingent upon the availability of nutritive substances. If the factor of animal resistance could be eliminated, the tumor would grow best in those organs where food is most abundant, but under ordinary circumstances, growth is modified to a greater or less extent by the local reaction, and this varies in different organs or tissues so that the picture actually presented in any given instance represents a resultant of the two factors. Thus, in the most fulminating cases of malignancy, cells may be distributed in large numbers to nearly every part of the body and they may grow almost anywhere, but they appear to grow best where food is most abundant. For example, in cases of this type, one almost invariably finds the most extensive growth in the liver. The lesions in other organs may be almost as numerous, but even in such organs as the kidneys they do not show the same activity of growth. In fact, they may be so small as to be barely perceptible. In the same way, metastases in the lungs, the skin, and subcutaneous tissues rarely showed a rate of growth equal to that of metastases in parenchymatous organs, lymph nodes, or muscle even though there was comparatively little local reaction to interfere with their nutrition.

In like manner, while cells are distributed both by the lymphatics and the blood, lymphatic distribution is a relatively slow process and any effect that it might have in determining the location of metastases may be forestalled to a great extent by a more rapid distribution of cells through the blood.

In certain instances, therefore, the development of metastases may become a function of time and of nourishment. This is especially true in animals with the most malignant tumors. Such animals are essentially indifferent; viable cells are distributed mechanically to all parts of the body, but on account of differences in the food supplied in different organs, certain lesions grow rapidly and death occurs before the cells in other locations have had an opportunity to develop.

In animals that show an increase in resistance with the progress of the disease, the distribution of cells and the growth of metastases are

determined more and more by positive factors of animal resistance. As long as resistance is comparatively low, cells may be widely distributed and growth may take place in many parts of the body, while the rate of growth is still determined largely by the nourishment provided in a given tissue. But in more resistant animals, or as resistance increases, the distribution of cells is more restricted and the actual food supply of an organ becomes of less moment. Under these circumstances, the important factors in the development of metastases are accessibility and availability which are determined on the one hand by the resistance encountered in the medium of cell transport, plus mechanical factors of distribution, and, on the other, by the local resistance to growth in different organs or tissues.

The effect of these conditions is to alter the general scheme of liability to metastatic involvement. Cell distribution tends to be limited more and more to those organs with the most abundant and direct blood supply or to parts that are accessible to the spread of the growth by the lymphatics, while a further restriction is imposed upon the development of metastases by the ability of the cell to grow in such places as are still accessible.

The first effect of these conditions is to produce a more slowly progressive disease with greater opportunity for the development of metastases, so that rapidly progressive cases of malignancy in animals that survive 6 or 8 weeks may show a more extensive distribution of metastases (gross) than those that die within the first 4 or 5 weeks, while the relative extent of the growth in different organs also undergoes some change. Thus, the growth in the kidneys was usually more abundant than that in the liver, and comparatively large lesions were found in a number of locations, while in cases of more acute death, they were either absent or extremely small. Hence in animals of this class, a more uniform distribution of lesions and a more uniform growth were the outstanding features of the picture presented as compared with that seen in the most fulminating cases of malignancy.

As the disease became more chronic, however, the order of metastatic involvement underwent a very decided alteration. The relative incidence of metastases in such organs as the lungs, the kidneys, the suprarenals, the liver, the heart, the eyes, the muscles, the superficial lymph nodes, the bones, the hypophysis, and the skin and subcutan-

eous tissues suffered a gradual or an abrupt reduction, the character and extent of the change varying with different organs. At the same time, there was an increase in the relative incidence of metastases in the deep lymphatics, in the retroperitoneal tissues, and in the mediastinum, and the change in one direction virtually compensated for that in the other. Finally, a terminal increase in the incidence and distribution of metastases occurred, so that in animals that succumbed after a prolonged course of disease, one might again find the same widespread distribution of lesions as in fulminating cases of malignancy, and this feature of the disease is deserving of especial emphasis.

Much of the change in the picture presented by these animals might be accounted for upon the basis of the principles of embolic distribution and what is known as to the ability of the cells to grow in different tissues. Such organs as the lungs, the kidneys, the suprarenals, and the liver still showed a fairly high incidence of metastases, and the decrease that occurred here or in other organs might be attributed to local tissue resistance. While this explanation might serve in the case of such organs as the skin and subcutaneous tissues, the lungs, the kidneys, and the liver, it would not hold in the case of the suprarenals, the hypophysis, the eyes, the bones, the superficial lymph nodes, and the muscles any more than it would in the case of the deep lymph nodes, the retroperitoneal tissues and the mediastinum where the incidence of metastases was upward rather than downward. In fact, there was no evidence of any local resistance to the growth of the tumor in either the suprarenals or the hypophysis, and the resistance encountered in the ciliary body, the lymph nodes, and the bones was comparatively feeble.

In view of the number of actively growing tumors in these animals, there can be no doubt that cells entered the blood stream and the lymphatics as in any other animals with actively growing tumors, but, for some reason, few of the cells entering the blood arrived at their destination in a viable condition or else they were removed from the circulating blood by some mechanism which interfered with their normal distribution. Under these circumstances, blood-borne metastases were, as has been said, confined to those organs with the most abundant and direct blood supply, while lymph-borne metastases either retained a comparatively high level of incidence or showed

an actual increase. Eventually a point was reached at which the growth appeared to spread almost entirely by the lymphatics, and few or no metastases were found, except in the retroperitoneal tissues and the mediastinum.

While in such animals as these the resistance to growth on the part of the tissues is doubtless greater than that of animals in which the disease pursues an acutely fatal course, it is obvious that conditions affecting cell transport play an even greater part in determining distribution and growth of metastases, for, as has been pointed out, there are still a number of organs in which the tumor might grow with ease provided the cells could reach such locations in a viable condition. This applies especially to the suprarenals and the hypophysis, where, as far as is known, there is virtually no local resistance and tumors never heal, except as a result of vascular occlusion. Moreover, as we have been able to show by direct inoculation, the suprarenals may still retain a degree of susceptibility even in a recovered animal.[2]

It is clear, therefore, that, in animals with a high native resistance or whose resistance increases at a rapid rate in consequence of a tumor inoculation, the incidence and distribution of metastases are primarily functions of cell distribution. In this instance, however, it is evident that we are no longer dealing with purely mechanical principles of cell embolism but with some positive factor of resistance analogous to that concerned in preventing the growth of cells injected intravenously into normal animals. That such is the case is shown by the fact that animals in which the disease has pursued a chronic course with metastases confined largely to lymphatic paths, may in the end develop numerous metastases in other parts of the body with showers of microscopic lesions in the vessels of such organs as the lungs, the kidneys, and the liver, indicating a gradual or sudden collapse of some mechanism which hitherto had prevented any extensive dissemination of viable cells by the blood. It will be recalled that this was the condition found in the animal with the spontaneous tumor.

If one pursues the line of reasoning that has been followed thus far it will be found that many of the phenomena of metastasis may be

[2] Pearce, L., and Van Allen, C. M., unpublished experiments.

explained upon the basis of the general principles of cell distribution and of growth which have been outlined above. As long as we are dealing with cases of high malignancy, the situation is comparatively simple, but as we pass from animals of this class to those that display a higher degree of resistance, we are confronted by increasing difficulties, and in any event, there are peculiarities of incidence and of distribution or of absence of metastases from certain organs for which it is difficult to find a satisfactory explanation. This applies to such conditions as the centralization of metastases, the low incidence of metastases in the uninoculated testicle, the spleen, and the thyroid and the complete absence of metastases from the central nervous system.[3] In all such instances, however, there is some evidence as to the nature of the cause that is responsible for the condition presented.

Attention has been called to the fact that metastases in general diminish in frequency with the distance from the body axis, or, where the blood is the medium of cell transport, other conditions being equal, they diminish with the distance from the heart. This indicates one of two things, either that the tumor cells tend to become lodged before they are carried very far or that the further they are transported, the less liable they are to reach their destination in a viable condition. In either case, it is obvious that they are subjected to some influence in the blood which tends to interfere with their growth, and the effects of this influence are more apparent under some conditions than under others. It is certain, however, that this is the main factor concerned in the centralization of metastases.

A similar condition appears to be responsible for the low incidence of metastases in the uninoculated testicle. The occurrence of so few metastatic lesions is difficult to reconcile with our knowledge of the growth of cells that are mechanically introduced, and it may be that the tortuous nature of the vessels and their mode of entrance into the testicle give an added protection against the entrance of tumor cells. This assumption is supported by the fact that metastases do occur with greater frequency in the cord and epididymis and that most of the cases of testicular metastases recorded were in animals with very malignant tumors.

[3] Since this statement was written, we have encountered one case of cerebral metastasis in which the growth apparently arose from the choroid plexus in the right lateral ventricle.

The problem presented by the thyroid is totally different. Persistent growth of tumor cells and the formation of visible metastases in the thyroid occurred only in animals whose resistance was of the lowest order. This cannot be accounted for on the basis of accessibility, since, with its abundant and fairly direct blood supply, it is reasonably certain that viable cells are constantly being brought to the thyroid in large numbers. Some of them make abortive attempts to grow but are almost immediately submerged beneath a massive chronic inflammatory reaction such as is not seen in any other organ or tissue. In fact, this reaction is maintained in some animals after it has practically failed elsewhere, and apparently it is the tangible means of suppressing tumor growth in this organ.

Neither of the above explanations is sufficient to account for the low incidence of metastases in the spleen. Tumor cells undoubtedly reach the spleen in large numbers, but for some reason they do not grow. There is no marked granulomatous reaction such as is believed to be a controlling factor in some organs, and it is equally difficult to attribute the immunity of the spleen to the presence of large amounts of lymphoid tissue, since the tumor metastasizes by way of lymphatics as well as the blood stream, or even when it cannot metastasize by the blood, and lymph nodes themselves are peculiarly favorable sites for the growth of the tumor. This, of course, is the paradox which renders it difficult to assign a rôle of especial importance to lymphoid tissues *per se* in combating such diseases as tuberculosis, syphilis, and cancer. In all these conditions, the causative agent of the disease lodges and grows in lymphoid tissues by preference, as it were, or as though it enjoyed a protection which was not afforded elsewhere, and, as is known, the causative agent of some of these diseases does survive longer in lymph nodes, spleen, and bone marrow than in other tissues. It is obvious, therefore, that the defensive function of these tissues can be exercised only in conjunction with other elements or tisssues. In other words, there are factors beyond the lymphoid tissues themselves.

In a few instances in which microscopic lesions were present in the spleen, the growth was located at the center of the Malpighian bodies. This may be no more than an indication of the point at which aggregates of cells are most apt to become lodged, or it may mean that the conditions for growth are better here

than in other parts of the spleen. While we do not pretend to know the exact causes for the failure of the cells to grow in the spleen, the evidence available would seem to indicate that anatomical or circulatory conditions are chiefly responsible in that the presence of a system of venous sinuses may afford an exceptional opportunity for the disposal of tumor cells.

The complete absence of metastases from the tissues of the central nervous system is still more perplexing. When inoculations are made directly into the substance of the brain, the tumor grows very actively and there can be no doubt that, with actively growing tumors in other parts of the body, large numbers of cells find their way into the capillaries of the central nervous system. Still no growth takes place. Hence it would seem that an especially efficient mechanism must bar their entrance to these tissues. This is not of the nature of a cellular reaction but apparently conditions exist which inhibit the growth of cells within the capillaries to a greater extent than in any other tissue of the body. The evidence available indicates that the mechanism concerned is the same as that which prevents the growth of cells that gain access to the blood stream of highly resistant animals but that its action is greatly intensified in the central nervous system.

It will be seen, therefore, that from whatever angle the problem of metastasis is approached, the crucial factor in determining widespread or restricted distribution of metastases or the presence or absence of metastases in any particular location is the factor of cell transport. The ability of the cells to grow is sometimes the decisive factor, but more often this is of secondary importance. The first line of defense is clearly the medium of cell transport, and in seeking an explanation for any given condition we are almost invariably forced to recognize the existence of some controlling mechanism in the blood or other body fluids whose action permits or prevents the occurrence of metastases on the one hand and regulates their distribution on the other.

The tumor may metastasize by the lymphatics when cells are incapable of surviving in the blood but, as long as the cells are distributed entirely by the lymphatics, the disease progresses extremely slowly and is stereotyped in form; variation in the picture presented, widespread metastases, and high malignancy, are largely functions of blood transport.

Thus far, no effort has been made to indicate the nature of the factor in the blood that regulates cell transport. We have dealt with animals in which this factor was either inoperative or only incompletely developed but we come now to a consideration of those animals that

show a complete absence of metastases. Once it has been shown that the cells of a malignant tumor are capable of surviving the process of transplantation and of growing in almost any part of the body, it is not so difficult to understand how cells from an actively growing tumor of a malignant character might gain lodgment in distant parts of the body and establish secondary foci of tumor growth as it is to understand why no secondary growths develop in other animals with tumors of the same character and equally active as to growth and local attributes of malignancy. This aspect of the subject is obviously of more far reaching importance than the presence of metastases and an understanding of one condition is essential to an understanding of the other.

It may be said at once that the reasons for the complete absence of metastases are not definitely known, but since in all animals there are numerous locations in which the tumor will grow with perfect ease, the failure of metastases cannot be accounted for on the basis of tissue susceptibility or local tissue resistance. There are, however, many facts which indicate that such a condition might be brought about by a reaction of the blood and of other body fluids. In approaching the problem from this point of view, one has first to consider the attitude of the animal toward the tumor cell as indicated by reactions which follow its introduction into the animal body. When cells are introduced into the tissues, they excite a reaction which is comparable in all respects to a foreign body reaction. There is an abundant growth of granulation tissue and an effort at encapsulation which may or may not succeed in checking the growth of the tumor, and subsequent phases of the reaction vary accordingly.

If the cells are injected into the peritoneal cavity, much the same thing happens. As has been stated, tumors rarely develop; the cells gradually become collected into small masses surrounded by fibrin and may remain free in the cavity for some time or become attached to the peritoneal surface and walled in by the growth of granulation tissue. In other words, the cells are disposed of as though they were some organized foreign material of a relatively indifferent character.

The fate of cells injected into the blood stream is not definitely known but the indications are that they are disposed of in much the

same way as cells that are introduced into the peritoneal cavity. At any rate, they rarely give rise to tumors, and since they are elements that are foreign to the blood, it is not unlikely that they tend to induce a formation of fibrin or of thrombi, such as Schmidt (7) has described in cases of human cancer, and are in this way prevented from obtaining the nourishment necessary for their growth.

If, therefore, we may be guided by the attitude of the animal toward the tumor cells, it is obvious that although they are products of rabbit tissues, the rabbit treats them as though they were foreign elements either because they have suffered some alteration which has affected their relations with normal tissues or because they are misplaced and brought into relations where they have no normal place. So long as we recognize the fact that the tumor cell arouses an antagonistic reaction, it is immaterial which of these explanations is accepted.

Again, if one analyzes this reaction in detail, it will be found that it presents many of the characteristics of an antigen-antibody reaction. This is seen in the increased resistance which occurs with the growth of the tumor, in the periodicity of growth and of resolution, and in the occurrence of the phenomenon which has been described as a crisis and reminds one strongly of a local anaphylactic effect.

In this connection, attention may also be called to the alteration which takes place in the coagulability of the blood and is intimately associated with clinical malignancy on the one hand and the incidence and distribution of metastases on the other. In fact, one of the most striking features of highly malignant tumors is the marked decrease in the coagulability of the blood which, in cases of fulminating malignancy, may extend even to incoagulability, while there is a change in the reverse direction in animals whose resistance is increased (3).

There is no evidence, however, that the reaction of the tumor animal is entirely specific. On the contrary, it is known that it is in part non-specific, but it is also known that resistance to the tumor may be greatly increased by non-specific stimulation.

The significance of these facts may be better appreciated if one applies them to the problem of metastasis, bearing in mind that conditions which favor or prevent the occurrence of metastases cannot be considered entirely apart from the primary tumor itself and from

processes which are brought into activity by its presence. As has been said, cells that are mechanically introduced into the circulating blood of normal animals are usually disposed of in such a way as to prevent their continued growth, and there is evidence that a similar reaction takes place when cells are discharged into the blood stream from a growing tumor. But, in view of the fact that the reaction (resistance) of normal rabbits differs with all forms of inoculation, it would not be expected that all animals would display the same sensitiveness to the presence of these cells or the same capacity for their disposal. At the beginning, therefore, other conditions being equal, the fate of the cells discharged into the blood would depend primarily upon the existing state of these two factors. With the continuation of the process, the cells and products of tumor growth acting as antigen, one of several conditions might be induced which may be illustrated by concrete examples of what actually does occur in different forms of the disease.

In the first place, many animals possess a high threshold resistance to the tumor cells in both the tissues and the body fluids. If, in addition to this, a gradual or rapid increase in the capacity for cell disposal occurred as a result of a process of automatic immunization, paralleling the reaction to the primary growth, then regression and absorption of the primary tumor would occur without the production of metastases.

In other animals, either with or without an increase in the native capacity for the disposal of cells, a sensitization might take place which would express itself in either or both of two ways: first, by a prompt disposal of cells discharged into the circulation, and second, by a kind of local anaphylaxis due to the action of antigen and antibody, if we may term them such, in the zone of concentrated antigen within and immediately surrounding the primary tumor. A condition of this kind does occur in many animals, giving rise to suppression of the primary growth by what has been termed a crisis. This condition, it will be recalled, is characterized by the sudden development of edema, which usually persists for a short time and then rapidly disappears. The essential element in this reaction appears to be the formation of coagula of some kind in the smaller vessels within or immediately surrounding the growth,

and possibly in the drainage lymphatics, which obstruct the flow of blood and lymph to a sufficient extent to cause an edema, and, while the obstruction is only temporary, it is usually sufficiently enduring to impair seriously the vitality of the growth and not infrequently produces a complete necrosis of the tumor. This reaction is identical in its obvious characters with that which occurs in syphilitic infections.

It is important to bear in mind that this type of reaction occurs with the most actively growing tumors, that while the time of occurrence varies with the rate of growth in different series of animals, it is remarkably frequent at or near the end of the 3rd week after inoculation. With a growth of this type, one would expect a maximum discharge of cells into the blood and lymphatics. Still, recovery frequently takes place without the development of metastases.

The exceptions to this rule are equally interesting. In some animals, the crisis is, as we have termed it, ineffectual, in that after partial regression, there is renewal of growth in the primary tumor, or, in spite of a critical reaction of considerable degree, there is little or no regression but the tumor continues to grow. In many instances, the growth of the primary tumor after crisis is comparatively slight, but the occurrence of an ineffectual crisis almost invariably signifies high or even fulminating malignancy which is the exact opposite of the condition which exists in the case of an effectual crisis. In such instances, it would seem that the animal expends a great part of its resources for preventing the successful implantation of cells. At all events, metastases appear in all parts of the body and grow with comparatively little opposition, indicating either that these animals possessed a feeble resistance from the beginning or that their mechanism of resistance to both cell transport and growth were affected at the same time and in the same manner.

This brings us to a third possibility as regards the behavior of animals towards the presence of tumor cells; namely, relative indifference with sluggish response. For a time, such animals would permit the growth of the tumor and the development of metastases in favorable locations, but as they became aroused, resistance would be increased and both metastases and primary tumor suppressed. In some instances, however, metastatic growths might remain in such places as the suprarenals or the eyes, and we would have the paradox of

an immune (?) animal with a living tumor which could not be suppressed but with other tissues protected either by the resistance in the medium of cell transport, or by a local tissue resistance.

The same conditions might be brought about by the existence of a relatively low threshold resistance with a reserve capacity sufficient to compensate for this defect. It is difficult to say whether the start that the tumor gets in such cases is due to indifference or to a low threshold resistance. In some instances, it appears as though the resistance of the animal were taxed to its uttermost, while in others, the tumor grows actively for a time but is checked abruptly and with no apparent effort on the part of the animal, which suggests that resistance was merely dormant.

A further possibility is that presented by the animal in which there is a persistence of an inactive or sluggish primary tumor with metastases distributed along the lymphatic paths of the body axis and after long periods of time gradually involving other organs of the body. The picture presented in these cases is clearly indicative of an initial metastasis by way of the lymphatics. This would imply an immunity to blood transport, since, when this path is open, metastasis is certain to occur by this route.

As has been pointed out, animals of this class not infrequently show a terminal systemic involvement which clearly indicates a collapse or exhaustion of the entire mechanism of resistance.

Finally, there is a group of animals that is either almost insensible to the presence of tumor cells or incapable of dealing with them either in the tissues or body fluids and the tumor progresses to a fatal termination with scarcely any opposition.

While, therefore, the ultimate cause may not be known for the absence of metastasis in animals in which it is reasonably certain that numbers of cells enter the blood and the lymphatics, it is highly probable that the conditions which have been outlined play a very considerable part in determining both the presence and the absence of metastases. In fact, it appears that the introduction of tumor cells into a normal animal acts in many ways as a parenteral injection of a foreign protein or of organized foreign material. But, in addition, the tumor cell is a living and aggressive agent which tends to perpetuate itself and in so doing arouses an antagonism on the part of the host

which, within the capacity of the animal, increases with the growth of the tumor or until one or the other prevails. This reaction is in many respects analogous to that seen in certain protozoan infections, which is not surprising since in both cases the causative agent is a highly organized animal cell.

If we adopt this attitude toward the tumor, it will be seen that the outcome of the interaction between tumor and host is determined by a chain of processes which are set in operation at the time of inoculation. Some normal animals possess the necessary capacity for suppressing the primary growth and at the same time are capable of disposing of any cells that may gain entrance to the blood stream or lymphatics. But, in most instances, the threshold resistance is comparatively low and increased powers of resistance are called into being by a process akin to immunization.

This reaction occurs in close association with the growth of the tumor but is directed against the tumor cells wherever they may be found. In like manner, the expression of resistance varies according to the means available for preventing the growth of cells in different locations. In all cases, however, there is an encapsulation in some form which deprives the cells of the nourishment necessary for their growth and at the same time hinders their distribution. Where viable cells gain lodgment in the tissues, a granulomatous reaction occurs and is aided in its action by the formation of coagula in the vessels that supply nourishment to these tumors. In the blood, tumor cells appear to be disposed of in the same way as any organized foreign material that cannot be phagocyted. They are surrounded by fibrin and filtered out of the circulating blood or are walled in by the formation of a thrombus.

Essentially the same thing occurs in the serous cavities and in the lymphatics, but the conditions which favor this method of disposal are not the same in all parts of the body. In some places, anatomical conditions are doubtless of importance, as in the case of the spleen; in others, the nature of the tissue aids in determining the end-result, as in the lungs and the skin on the one hand, where growth is difficult even under the most favorable circumstances, and in the liver and kidneys and the suprarenals, the hypophysis, the lymph nodes, the bone marrow, and the eyes, on the other, where food is either

abundant or the local resistance to tumor growth is comparatively feeble.

Finally, it is clear that in certain organs, such as the central nervous system, there is an additional factor which aids in the disposal of cells in the circulating blood. It is known that the tissue of the central nervous system contains substances (cephalin) which are among the most powerful coagulants, and it appears not unlikely that the freedom of certain organs from metastases may be attributable to the action of some substance which facilitates coagulation about the cells contained in the blood passing through these organs.

There may be other processes concerned in the disposal of tumor cells, but there is no evidence to indicate that such is the case and the known facts do not require such an assumption.

Little is known of processes of protection or immunity involving phenomena of coagulation such as appear to be operative with this tumor. The phenomena described are analogous to what has been observed in so called agglomeration reactions of protozoa (trypanosomes and blood spirochetes), and it is not improbable that similar processes may be concerned in freeing the blood of foreign cells or organisms of many kinds.

Pathologists have realized that some humoral factor must play a part in the resistance to malignant tumors and to protozoan diseases, but attempts to demonstrate protective principles have been confined so largely to studies of serum, as distinguished from plasma or whole blood, that the importance of phenomena of coagulation has not been fully appreciated.

It is not known how the changes in coagulation are effected, but there are a number of possibilities to be considered. Among these are the loss of the calcium that occurs in malignant disease and variations in fibrinogen and thrombokinase, such as occur in practically all forms of parenteral injection of foreign proteins, both of which have a direct bearing upon the problem of tumor resistance. However, it will require further investigation to determine the nature of the change that at one time enables the blood to render inactive all the cells that enter and at another leads it to behave as an inert medium distributing the cells to different parts of the body in a purely mechanical way.

CONCLUSIONS.

With a knowledge of what takes place at the site of inoculation, in the medium of cell transport, and at the point of lodgment of cells in different parts of the body, the problem of metastasis is greatly clarified. It is possible to explain both the presence and the absence of metastases as well as peculiarities of incidence and of distribution upon the basis of established facts which in no wise conflict with what has been determined in the case of other tumors. On the contrary, if this conception be adopted, it will aid materially in clarifying our understanding of phenomena of metastasis or of tumor growth in general.

Once it is granted that the tumor cell is capable of exciting an antagonistic reaction on the part of the host, either in virtue of a change in the cell itself or of its entrance into an environment where it has no normal place, the problem immediately resolves itself into a consideration of the consequences entailed in accordance with the general scheme that has been outlined above.

When viewed from this standpoint, the current conception of a malignant tumor as a lawless growth (autonomous) is hardly tenable. It may be viewed as an abnormal growth resulting from a disturbance of a growth-regulating mechanism and is lawless in the sense that it tends to override existing processes of restraint, but its behavior is that of a living and aggressive foreign agent, and all the phenomena associated with its presence are susceptible of analysis upon the basis of the same principles of pathological action that are applicable to such conditions. Even the distinctions that are recognized in the mode of metastasis of different classes of tumors are explainable upon this basis, not as expression of any inherent tendency on the part of the tumor to spread by any particular path but merely as results of following the line of least resistance. Naturally this would vary according to the origin of the cell. Thus cells of a sarcoma are not so far removed genetically from blood elements as are epithelial cells and hence might arouse less antagonism if they entered the blood stream and, under ordinary circumstances, would be more likely to be distributed by this path than the cells of a carcinoma. On the other hand, metastasis by way of the lymphatics is probably not due to any selective

activity but is a matter of necessity in many instances, or until the blood resistance has been reduced to such an extent that cells entering the blood stream are no longer destroyed.

This discussion of the phenomena of metastasis leaves many questions still untouched. The foremost of these are the causes for the wide variations in the incidence of metastases (28 to 100 per cent) in different series of animals and for such differences in the behavior of individual animals as have been described. These subjects have a more intimate connection with conditions that affect malignancy or animal resistance and will be taken up in the next papers of this series which deal with variations in malignancy and factors that influence animal resistance.

SUMMARY.

Data bearing on the occurrence and distribution of metastases, as recorded in the preceding papers of the series, are analyzed with reference to the parts played by the tumor cell and by conditions which influence cell distribution and cell growth.

It is shown that, while the tumor cell is an essential element in the production of metastases, its influence is relatively constant, and that the prime factors responsible for variations in the occurrence and distribution of metastatic growths are conditions that influence cell distribution or that affect the viability of the cell during transport, and conditions that affect the nutrition of the cell wherever it may become lodged.

It is further shown that both the presence and the absence of metastases, as well as peculiarities of distribution and of growth, are susceptible of a comparatively simple explanation upon the basis of well recognized principles of pathological action. In brief, it was found that, while the tumor cells might be distributed by either the blood stream or the lymphatics, the mode of distribution and the fate of the cells were determined in accordance with the same general principles that are applicable to the distribution and disposal of foreign cells introduced into the animal body. It was pointed out, however, that in applying these principles to tumor metastasis, it was necessary to recognize the fact that tumor cells bear a definite relation to the tissues of the host and that they are living and agressive agents. Hence, the

reaction that they arouse differs in some respects from that produced by inert or unorganized foreign material or by living cells of a foreign species.

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A METHOD FOR THE PHYSIOLOGICAL STUDY OF TISSUES IN VITRO.

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PLATES 30 AND 31.

(Received for publication, May 21, 1923.)

The aim of the method described in the present paper is to maintain tissues in a condition of uninterrupted growth in a medium which does not deteriorate spontaneously. This simple idea has doubtless occurred to many experimenters, but great technical difficulties have so far prevented the development on this principle of a method of cultivating tissues. The problem consists of giving the cells the necessary food material and removing the catabolic substances from the medium without disturbing the tissues and without bacterial contamination. It has been solved by the construction of containers and instruments permitting the aseptic handling of the cultures, and by the use of a medium composed of two parts, solid and fluid, the solid medium being continually bathed by the fluid medium which is changed as often as necessary.

I.

Preparation and Handling of the Cultures.

1. Containers and Instruments.—The containers are flat, round flasks with narrow, oblique necks through which tissues and media may be introduced and removed. The neck is 3 cm. long and 1 cm. wide, and can be easily sterilized in a Bunsen flame. The width of the neck and its inclination are such that tissues and fluids are easily introduced with a pipette, and particles of dust cannot fall into the culture medium while the flask is opened. It is closed with absorbent cotton and a rubber cap. The flasks belong to five types (Fig. 1), and each type may be made in two sizes, 5 cm. or 8 cm. in diameter.

Type A bears a top opening 3 cm. in diameter, and is used for the cultivation of tissues which cannot be handled conveniently through the neck of the flask. The opening is closed by a disc of glass, fixed with shellac. Type B has two necks at the opposite sides of equal diameter and no top opening. It is convenient when the preparation of the culture requires the handling of the tissues with two instruments, which are introduced through the opposite necks. Type C has a long neck, a bottom opening closed by a thin mica plate, and also a top opening. It may easily be inverted under a microscope for photography or examination under high power of the living tissues. After the culture has been fixed in formaldehyde, the bottom of mica is excised with ordinary scissors or unsealed without disturbing the solid medium. Then the tissues and their medium, adherent to the thin mica plate, may be stained and studied under high magnification. Type D has only one neck and is commonly used for the cultivation of fibroblasts, epithelium, leucocytes, and virus, and for the study of cell secretions. Type E resembles Type D, and possesses a bottom opening covered with mica. Flasks A, B, and D are made of plain glass which allows the tracing of the cultures in a projectoscope and also low power microscopic examination. When cytological studies are required flasks of Types C and E are used.

The tissue fragments are handled and introduced into the flask with long platinum spatulas, straight or curved, and mounted on a glass rod (Fig. 2). Other kinds of spatulas are used for cutting the solid medium, when part of the culture has to be removed from the flask. The introduction of the fluids into the flask is made with short, graduated pipettes with rubber nipples (Fig. 3). The fluids are removed through a large hollow needle 4 cm. long, connected by rubber tubing with a vacuum apparatus (Fig. 3). The aspirating needle is also used when aeration of the culture is needed.

2. Culture Medium.—The culture medium is composed of two parts, solid and fluid. The solid medium consists of a coagulum of fibrin obtained from plasma or fibrinogen. When fibrinogen forms the basis of the clot, the fibrin is protected from digestion by a little serum or, when serum cannot be used, by a small amount of sodium linoleate or suspension of egg yolk. The volume of the solid medium in a flask 5 cm. in diameter should be 2 cc. If it is larger, the coagulum

becomes too thick and the tissues are isolated from the fluid medium by a layer of fibrin through which the diffusion of nutrient and catabolic substances is very slow. The volume of the fluid medium is generally 1 cc. The asepsis of the fluids used in the composition of the medium must be ascertained by the proper bacteriological tests, and the H ion concentration by the colorimetric method.

The plasma is introduced first. Generally 0.5 cc. of plasma is injected into the flask, which is gently tilted in order that the whole bottom may be moistened. This prevents the loosening of the coagulum from the glass surface, which may otherwise occur after a few days. Then 1.5 cc. of Tyrode solution, containing 5 per cent tissue extract, is introduced and mixed with the plasma. When a fibrinogen suspension is used instead of plasma, it is introduced in the same manner. To 0.5 cc. of fibrinogen suspension is added 0.5 cc. of doubly concentrated Tyrode solution. Then 0.5 cc. of Tyrode containing a little sodium linoleate or serum, and 0.5 cc. of dilute embryonic tissue extract, are introduced. Before coagulation takes place, the fragments of tissues, carried on a platinum spatula, are placed in the medium. If there are many fragments, they are suspended in Tyrode solution and injected with a pipette. After coagulation has taken place, the fluid medium is poured on the surface of the clot, and the neck of the flask is flamed and tightly closed.

The fluid medium must be changed every 2nd, 3rd, 4th, or 5th day, according to the nature of the medium and the tissues. The flasks are brought into a room where the air has been sprayed and is practically free of dust. The rubber caps are removed and the neck is carefully flamed. Then the fluid is withdrawn by means of the aspirator (Fig. 3) or a pipette, and the new fluid introduced. The neck is again flamed and closed. The time required for changing a complex medium varies from 45 to 75 seconds. It is generally possible to handle about 60 flasks in 1 hour. If a fragment of the culture has to be transferred to another flask, the coagulum is detached with a spatula and removed through the neck of the flask. Then the tissues are dissected and a new culture is made.

3. Measurement of the Rate of Growth and Examination of the Cells.— The flasks are placed in a projectoscope and the outline of the tissue is traced every 1 or 2 days. As the thickness of the solid medium and

the density of the fibrin meshwork are about uniform, the increase in area of the tissue expresses the increase of its volume with some accuracy. When the area is plotted in ordinate and the time in abscissa, the curve expressing the growth of connective tissue or of epithelium is a parabola.

Microscopic examination of the tissue can be made with low power through the bottom of Flasks A, B, and D, and with high power through the mica sheets of Flasks C and E.

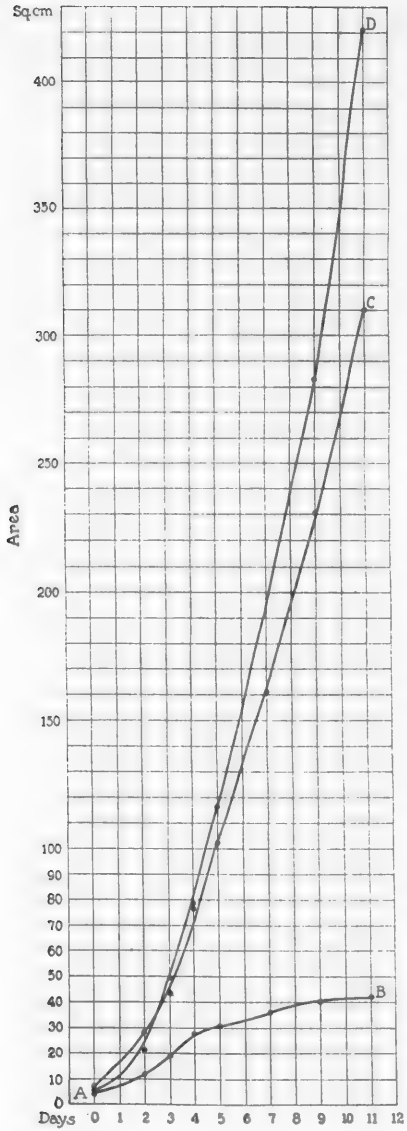
The tissues are fixed, the flask being still in the incubator, by warm Ringer solution containing 2 per cent formaldehyde. After fixation, the culture is washed and detached from the flask, then stained, or sectioned and stained. But the tissues are often damaged in the process of removal from the bottom of the flask. Therefore, when microscopic examination is needed, the tissues are grown on the bottom of Flasks C and E.

II.

Characteristics of the Growth.

The fibroblasts were obtained from an 11 year old strain of connective tissue, or from the heart of chick embryos. They were imbedded in the coagulum covering the bottom of the flask and bathed in the fluid medium. When the fluid medium was composed only of Tyrode solution or serum, the fibroblasts used the food material stored in the tissue fragment itself. Then, the rate of growth became slower and ultimately the tissues died. The duration of the life of the culture in this non-nutrient medium expressed the residual activity of the tissue and its graphic representation is generally a long S-shaped curve (Text-fig. 1, Curve *AB*). When the fluid medium contained some nutrient substances, the tissues increased regularly in mass (Fig. 4). The diameter of the new colony might reach 1.8 or 2 cm. after 2 or 3 weeks, and the curve of the growth was a parabola (Text-fig. 1, Curves *AC* and *AD*).

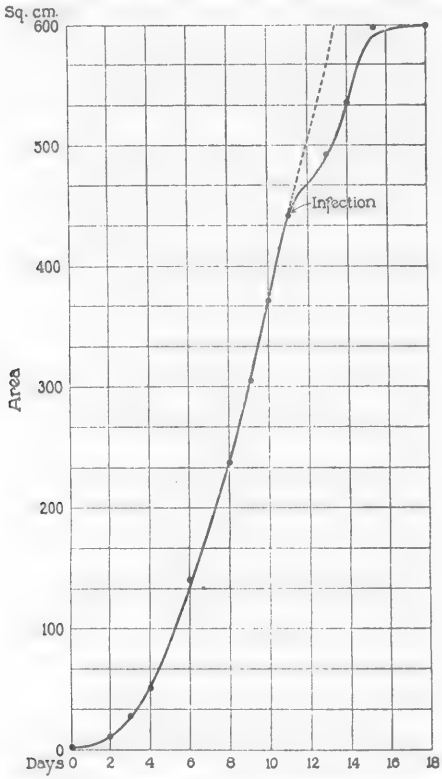
The rate of growth was modified by minute changes in the constitution of the medium. When bacterial contamination occurred, the rate of growth decreased and the tissues died (Text-fig. 2). Slight differences in the composition of the medium brought about marked differences in the rate of growth. During the first days of life of the



TEXT-FIG. 1. Curve *AB*, growth of fibroblasts in a medium containing no nitrogen. Curve *AC*, growth of fibroblasts in a medium containing 21 mg. of nitrogen per 100 cc. Curve *AD*, growth of fibroblasts in a medium containing 30 mg. of nitrogen per 100 cc.

cultures, these differences were not apparent, but after 2 or 3 weeks, they became evident (Text-fig. 1).

The changes in the rate of growth of a given tissue under the influence of another tissue could also be studied. For instance, when a colony of leucocytes, growing under the influence of serum, reached



TEXT-FIG. 2. Growth of a culture of fibroblasts which became infected on the 11th day and ultimately died.

the edge of a resting culture of fibroblasts, these cells began to proliferate again.

Epithelial cells grew as a membrane at the surface of the coagulum. The curve of growth was also a parabola. The rate of growth of epithelium was always less rapid than that of fibroblasts.

Leucocytes from the blood and wandering cells from the spleen were cultivated in Flasks D and E. From spleen fragments, about 1 sq. mm. of wandering cells migrated and in 30 or 35 days covered an area of about 2,000 sq. mm.; that is, the entire medium. Leucocytes from small blood clots grew closely packed together and later formed a number of small colonies scattered through the medium. These colonies originated by the grafting on the surface of the coagulum of groups of cells which floated into the fluid medium. They never aggregated in a tissue, and an accurate measurement of the area invaded by them was not possible.

III.

Accidental Causes of Death.

When the solid medium remains unaltered, and the proper nutrient fluid is added to it from time to time, the tissues invade the coagulum progressively. But several factors may interfere with their growth and bring about premature death. The more important of these factors are bacterial contamination, changes in the H ion concentration of the medium, and alteration of the coagulum.

The first is generally due to the contamination of the fluids composing the medium during their preparation; to non-sterile instruments, pipettes, or spatulas; to contact between the fluid medium and the stopper of the flask, and finally to contamination from the air. Plasma is never contaminated because, being received directly into tubes, it is not exposed to air and contaminating contacts in the course of its preparation. As Tyrode solution is sterilized by filtration through a Berkefeld filter, it is almost never a source of contamination. Tissue juice is often responsible for contamination. However, the following technique usually yields an aseptic preparation. The embryos are extirpated carefully from the egg or from the uterus, and immediately introduced into a Latapie apparatus. Then, the pulp is received in a graduated flask and the necessary amount of Tyrode solution is added. After centrifugation, the extract is placed in a number of small tubes in order that the contents of each tube may be used entirely, after the tube is opened. The condition of each tube must always be tested by cultivation of a sample of fluid in bouillon

and on agar. As some bacteria develop very slowly, these cultures have to be kept under observation for 5 or 6 days, before the fluids can be considered as sterile and used for the growth of tissues. Practically all the failures could be traced to a contamination of the tissue juice which had been overlooked in the examination of the bacterial cultures. The opening of the flasks, the aspiration of the fluid, and the introduction of new medium practically never give rise to contamination, because the neck of the flask and the spatulas are easily sterilized in a Bunsen burner flame, and the pipettes are easily kept aseptic. Contamination may also be traced to contact of the fluid medium with the cap when the flasks are handled without sufficient care. None occurs as long as the fluid medium does not penetrate the oblique neck during the transportation of the flasks. When the flasks are carried in a tray especially constructed, where they are placed vertically, with the neck up, there is no contact of the fluid with the stopper, and the number of bacterial contaminations is much lessened.

The infection by air appears to be very unusual. In the room where the flasks are opened and the fluid medium is replaced, the number of bacterial colonies found in half an hour on Petri dishes about 100 sq. cm. in area is only 3. As the section of the neck of the flasks is less than 1 sq. cm. and the time during which the flask is opened does not exceed 30 seconds, the chances of contamination by air are extremely small. Besides, if a few particles of dust should fall inside the neck, they would be destroyed by the flaming which always precedes the closing of the flask. When the air contained in the flasks is aspirated and replaced by the air from the room, contamination is also quite exceptional.

Another cause of death of the tissues is a change in the reaction of the medium. When the pH of the medium is higher than 8, the growth decreases and sometimes ceases entirely. The excess of alkali may come from the glass. Therefore, every new flask has to be tested from this point of view before being used. Generally the alkali comes from insufficient washing. A special apparatus has been made for rinsing the flasks after they have been cleansed with sodium hydroxide. Since its use, no losses due to excess of alkali have occurred. A more frequent source of accident is acid formation.

When the pH of the culture is lower than 7.2, the rate of growth is markedly modified and the tissues die. This is generally due to a spontaneous change of the tissue extract which occurs mainly when particles of tissues are suspended in the fluid. The H ion concentration of every fluid used in the cultures must be tested and adjusted to 7.6 or 7.8.

When the fluid medium is composed only of tissue juice, the fibrin may become partly digested after a few days. This accident is prevented by adding to the tissue juice a small amount of serum, which protects the fibrin against the proteolytic enzymes. Sodium linoleate or egg yolk may be substituted for serum. When the beginning of the digestion of the fibrin is observed, a few drops of plasma are placed at the surface of the coagulum and the tissues generally resume their normal growth.

IV.

DISCUSSION.

The elucidation of the fundamental relations between the tissues and the humors of the organism would require an analysis of the action of the serum constituents on pure strains of cells, of the effect of the secretions of those cells on the composition of serum, and of the influence of the tissues upon one another. The main requisites of a method permitting such an investigation are to maintain pure cultures of cells in a condition of uninterrupted growth in a medium of practically unchanging composition. We already possess techniques by which strains of fibroblasts,[1] epithelial cells,[2] and leucocytes[3] may be isolated and kept free from contamination by other cells. But these tissues must be maintained in a known condition; that is, in a medium which does not deteriorate spontaneously. As the activity of a tissue is a function of the concentration of certain substances in its medium,[4] it is evident that the effect of a given factor on cell proliferation or morphology cannot be ascertained if at the same time the medium undergoes spontaneous and ill defined modifications.

[1] Ebeling, A. H., *J. Exp. Med.*, 1922, xxxv, 755.
[2] Fischer, A., *J. Exp. Med.*, 1922, xxxv, 367.
[3] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxvi, 365.
[4] Carrel, A., *J. Exp. Med.*, 1913, xviii, 287.

None of the techniques used so far fulfill these two main conditions. In the early method of so called cultivation of tissues, which is still employed by many experimenters, a fragment of fresh tissue, generally embryonic, is placed in a drop of plasma[5] or saline solution,[6] and for a few days migration and multiplication of cells may be observed. But the phenomenon is irregular and of short duration, and no increase in the mass of the tissues is observed. When the area of a pure culture of fibroblasts so cultivated is accurately measured, the curve of the growth resembles that of a unimolecular autocatalytic reaction. The second inflection of the S-shaped curve begins after 48 hours,[7] and is due to a change of the medium which greatly restrains the rate of growth and brings about the death of the cells. During 48 hours only, the growth is uninfluenced by the progressive deterioration of the medium. Later, the action of a given factor on the growth of fibroblasts cannot be studied on account of the simultaneous effect of the modifications of the medium on the tissues.

In order to prevent the effect of the deterioration of the medium, a method was developed long ago[8] in which the tissues were removed from the medium after 48 hours, washed in Ringer solution, and placed in a new medium. By the continual transfer of the tissues from medium to medium,[1] the cells were maintained in an identical condition, and the rate of growth of a strain of connective tissue, cultivated in this manner for more than 11 years, has not varied.[1] Today these fibroblasts multiply with the same velocity as 10 years ago. Many physiological problems have been investigated with the help of this technique. But it cannot be applied so effectively to more delicate structures such as epithelium and leucocytes. The amount of tissue is very small and the presence of secretions cannot be ascertained. Moreover, the section of the tissues and their transfer require a great deal of training and manual skill.[9] As the growth is interrupted every 48 hours, the prolonged action of a given factor must

[5] Carrel, A., and Burrows, M. T., *J. Exp. Med.*, 1911, xiii, 387.

[6] Lewis, M. R., *Anat. Rec.*, 1915–16, x, 287. Lewis, M. R., and Lewis, W. H., *J. Am. Med. Assn.*, 1911, lvi, 1795.

[7] Ebeling, A. H., unpublished experiments, 1919.

[8] Carrel, A., *J. Exp. Med.*, 1912, xv, 516.

[9] Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 231.

be observed during a number of passages. Thus, it involves much labor, and the process of growth cannot be expressed graphically in a satisfactory manner. For these reasons, an attempt was made to develop a better method.

It seems that the technique described in this article, although far from perfect, may be applied to the solution of many problems and fulfill most of the conditions required for a physiological study of tissues *in vitro*. However, the medium deteriorates in some measure because the substances contained in the fibrin do not diffuse entirely into the fluid which covers its surface. The coagulum, incompletely washed by the fluid medium, may be slowly modified by the substances which progressively accumulate in its meshwork. But the parabola expressing the increase of the tissues indicates that the growth proceeds at a constant rate for 2 or 3 weeks, instead of 2 days as in the hanging drop technique. A culture of fibroblasts or of epithelium can be kept in a D flask for about 3 weeks in a condition of active life. This space of time is longer than necessary for the study of most physiological phenomena. Leucocytes can remain in excellent condition for at least 35 days,[10] and the coagulum is practically as transparent after this period of time as on the 1st day, although it may be completely invaded by the wandering cells. As fifteen or twenty fragments of tissues or of leucocytic film can be placed in a D flask containing 2 cc. of medium, the nature of cell secretions can be investigated in some measure.

The growth, being uninterrupted, may be represented graphically, and the part played by the constituents of the medium in the growth analyzed without difficulty. The characters of the curve vary according to the nature of the medium. In a nutrient medium the growth is expressed by a parabola and in a preservative medium by an S-shaped curve. This curve is the measure of the residual activity of a tissue. It is known that the activity of a tissue at a given instant is function of at least three independent variables, the inherent cell activity at the preceding instant, and the concentrations of growth-activating and growth-inhibiting substances in the medium. The inherent activity of a tissue may be defined as that which would be displayed during an instant in a medium deprived of all nutrient substances. The energy used by a tissue in that condition would be supplied by the

[10] Carrel, A., unpublished experiments, February, 1923.

food material stored in the cells or in the interstitial lymph, and not by the medium. This inherent activity cannot be measured directly. But it is certainly proportional to the residual activity; that is, to the increase in area of the tissue and to the duration of its life in a preservative non-nutrient medium. The residual activity being known, it is easy to measure the part which is played in the process of growth by the nutrient substances of the medium. The area *ABD* in Textfig. 1 represents the increase of the tissue due to the action of the medium; that is, the amount of protoplasm built up by the cells from the nutrient substances contained in the medium.

There is no doubt that this method permits the measurement, conveniently and without great labor, of the effect of many factors on cell proliferation, and will therefore help in the study of physiological and pathological problems.

V.

CONCLUSIONS.

1. A method has been developed which allows the continuous growth of pure strains of fibroblasts, epithelium, and leucocytes in a medium which undergoes but slight spontaneous deterioration.

2. The principle of the method is to leave the tissues undisturbed while the medium is changed. This was realized by special containers allowing the change of the medium without bacterial contamination and by the simultaneous use of a solid and a fluid medium.

3. The curve of growth of pure cultures of fibroblasts and epithelial cells in a nutrient medium is a parabola; in a non-nutrient medium, it is S-shaped and expresses the residual activity of the tissues. Leucocytes invade the culture medium progressively, as do bacteria, but never aggregate in a tissue.

4. The method is used for the study of the morphological and dynamic changes occurring in tissues under the influence of chemical and physical factors.

EXPLANATION OF PLATES.

PLATE 30.

FIG. 1. Flasks A, B, C, D, and E.

FIG. 2. Introduction of tissues into a D-5 flask with a spatula.

PLATE 31.

FIG. 3. Aspiration of the fluid.

FIG. 4. Culture of fibroblasts in a D flask.

FIG. 1.

FIG. 2.

(Carrel: Physiological studies of tissues *in vitro*.)

FIG. 3.

FIG. 4.

(Carrel: Physiological studies of tissues *in vitro*.)

ANTAGONISTIC GROWTH PRINCIPLES OF SERUM AND THEIR RELATION TO OLD AGE.

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(Received for publication, May 21, 1923.)

I.

INTRODUCTION.

The restraining effect of serum on homologous fibroblasts can be attributed to the antagonistic action of growth-activating and growth-inhibiting substances, the effect of the inhibiting substances always being more pronounced than that of the activating substances.[1,2] The activating substances are thermolabile[3] and accompany the precipitate obtained by bubbling carbon dioxide through diluted serum.[2] They are probably of the same nature as the growth-activating substances contained in embryonic tissue juice[4] and leucocytic extracts or secretions,[5] and may be considered as the product of gland and leucocytic activities.[6] The inhibiting substances resist heat at 65°C.,[3] and remain in the serum with the albumin fraction.[2]

It may be supposed, therefore, that the enhanced restraining action of serum in old age is caused by a decrease of the activating substances, or by an increase of the inhibiting substances, or by both. In order to test this hypothesis, the effect of heating the sera of young and old animals at 65°C., and of precipitating it by carbon dioxide, was studied. If the serum of an old animal contains a lesser amount

[1] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 599; 1922, xxxvi, 399.

[2] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1923, xxxvii, 653.

[3] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxv, 647.

[4] Carrel, A., *J. Exp. Med.*, 1913, xvii, 14.

[5] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxvi, 365. Carrel, A., *J. Exp. Med.*, 1922, xxxvi, 385.

[6] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxvi, 645.

of thermolabile, activating substances than that of a young animal, the inhibiting action of the heated serum of a young animal should be relatively more enhanced than that of an old animal. At the same time, the substances precipitated from the serum of an old animal by carbon dioxide should not stimulate the multiplication of fibroblasts so much as the substances obtained from the serum of a young animal.

II.

Action on Homologous Fibroblasts of the Heated Sera of Young and Old Chickens.

The serum obtained from the plasma of chickens 10 months and 6 years old was heated for 1 hour at 65°C. Its inhibiting action was

TABLE I.

Rate of Growth of Homologous Fibroblasts in Serum, Heated at 65°C. for 1 Hour, from a 10 Month Old Chicken.

Group No.*	Culture No.	Rate of growth in serum.		Ratio: $\frac{E}{C}$.	Serum.			
					Original.		Heated.	
		Unheated (*C*).	Heated (*E*).		pH	Refractive index.	pH	Refractive index.
1	31852	3.61	2.15	0.60	8.0	1.3354	7.8	1.3353
2	31853	3.55	2.23	0.63	8.0	1.3354	7.8	1.3353
Average.....				0.62				

* Each group was composed of four experiments.

TABLE II.

Rate of Growth of Homologous Fibroblasts in Serum, Heated at 65°C. for 1 Hour, from a 6 Year Old Chicken.

Group No.*	Culture No.	Rate of growth in serum.		Ratio: $\frac{E}{C}$.	Serum.			
					Original.		Heated.	
		Unheated (*C*).	Heated (*E*).		pH	Refractive index.	pH	Refractive index.
1	31854	1.95	1.67	0.86	8.0	1.3370	8.0	1.3370
2	31855	1.95	1.60	0.82	8.0	1.3370	8.0	1.3370
Average.....				0.84				

* Each group was composed of four experiments.

ascertained by comparing the rate of growth of fibroblasts obtained from an 11 year old strain in media containing 50 per cent unheated or heated sera.

In two groups of four experiments, the effects of unheated and heated sera of young and old animals were compared. After being subjected to heat, the sera of the young chickens became 38 per cent more inhibiting, while that of the old chickens became only 16 per cent more inhibiting (Tables I and II).

There was no doubt that the action of heat increases the inhibiting action of the serum of a young animal comparatively more than that of an old animal. Therefore, it may be assumed that in old age the

TABLE III.

Rate of Growth of Homologous Fibroblasts in Sera from a 10 Month and a 6 Year Old Chicken.

Group No.*	Culture No.	Rate of growth in serum.		Ratio: $\frac{E}{C}$	Serum.			
					Young.		Old.	
		Young (*C*).	Old (*E*).		pH	Refractive index.	pH	Refractive index.
1	31756	3.74	1.96	0.52	8.2	1.3370	8.2	1.3390
2	31808	3.29	2.29	0.70	8.2	1.3370	8.2	1.3390
Average.....				0.61				

* Each group was composed of four experiments.

serum contains relatively less thermolabile growth-activating substances than in youth.

Then it was sought to ascertain whether the heated serum of an old animal is still more inhibiting than that of a young animal, or whether the inhibiting power has become similar. In two groups of four experiments, the action of the serum of a young animal was compared to that of an old animal. The rate of growth of fibroblasts was 39 per cent slower in the serum of the old animal than in that of the young one (Table III). The same experiment was repeated after the sera had been heated, and the inhibiting action of the serum of the old animal was still 24 per cent greater than that of the young animal (Table IV). Then, the increased inhibiting action of serum in old age cannot be considered as due only to the disappearance of thermo-

labile, activating substances, but also to a more marked effect of the inhibiting principles.

TABLE IV.

Rate of Growth of Homologous Fibroblasts in Sera, Heated at 65°C. for 1 Hour, from a 10 Month and a 6 Year Old Chicken.

Group No.*	Culture No.	Rate of growth in heated serum.		Ratio: $\frac{E}{C}$.	Serum.			
					Young.		Old.	
		Young. (*C*).	Old. (*E*).		pH	Refractive index.	pH	Refractive index.
1	31838	2.18	1.73	0.79	7.8	1.3353	8.0	1.3370
2	31843	2.17	1.59	0.73	7.8	1.3353	8.0	1.3370
Average.....				0.76				

* Each group was composed of four experiments.

TABLE V.

Action of CO_2 Precipitate, from Sera of Young Chickens, on the Rate of Growth of Homologous Fibroblasts.

Group No.*	Culture No.	Age of animal.	Rate of growth in.		Ratio: $\frac{E}{C}$.	Tyrode solution.		Precipitate solution.	
			Tyrode solution (*C*).	Precipitate solution (*E*).		pH	Refractive index.	pH	Refractive index.
		mos.							
1	31689	10	9.34	9.75		7.8	1.3285	7.8	1.3310
2	31702	10	4.84	5.83	1.19	7.8	1.3285	7.8	1.3310
3	31783	10	4.37	5.15		7.8	1.3285	7.8	1.3300
4	31800	10	4.62	5.34	1.17	7.8	1.3285	7.8	1.3300
5	32142	3	4.92	6.12		7.8	1.3279	7.8	1.3291
6	32154	3	4.63	5.54	1.22	7.8	1.3279	7.8	1.3291
		yr.							
7	32103	1	5.01	5.82		7.8	1.3280	7.8	1.3289
8	32114	1	6.58	7.74	1.17	7.8	1.3280	7.8	1.3289
Average.....					1.19				

* Each group averaged from two to four experiments.

III.

Action on Homologous Fibroblasts of the Carbon Dioxide Precipitate Obtained from Sera of Young and Old Animals.

The sera from 3 month, 10 month, 3 year, and 6 year old chickens were precipitated by carbon dioxide according to a technique previously described.[2] The precipitates were dissolved in Tyrode solution and the effect of the respective solutions on homologous fibroblasts was compared to that of Tyrode solution.

TABLE VI.

Action of CO_2 Precipitate, from Sera of Old Chickens, on the Rate of Growth of Homologous Fibroblasts.

Group No.*	Culture No.	Age of animal.	Rate of growth in.		Ratio: $\frac{E}{C}$.	Tyrode solution.		Precipitate solution.	
			Tyrode solution (*C*).	Precipitate solution (*E*).		pH	Refractive index.	pH	Refractive index.
		yrs.							
1	31784	3	4.60	4.38		7.8	1.3285	7.8	1.3300
2	31801	3	4.61	4.53	0.97	7.8	1.3285	7.8	1.3300
3	32123	3	5.51	6.06	1.10	7.8	1.3280	7.8	1.3285
4	31690	6	11.05	11.23		7.8	1.3285	8.0	1.3310
5	31703	6	5.33	5.14		7.8	1.3285	8.0	1.3310
6	32102	6	4.86	5.13		7.8	1.3280	7.8	1.3290
7	32113	6	5.87	5.99	1.01	7.8	1.3280	7.8	1.3290
Average.....					1.03				

* Each group averaged from two to four experiments.

The growth of fibroblasts in 48 hours was 16 per cent larger in the precipitate solution of the serum of the young animals than in Tyrode solution (Table V), while the activity of the precipitate solution from the serum of the older animals was about identical with that of the Tyrode solution (Table VI). This was a direct proof of the disappearance of the growth-activating substance of serum during the course of life.

TABLE VII.

Action of Serum, after Removal of CO_2 Precipitate, from Young Chickens, on the Rate of Growth of Homologous Fibroblasts.

Group No.*	Culture No.	Age of animal.	Rate of growth in serum.		Ratio: $\frac{E}{C}$.	Serum.			
						Original.		Treated.	
			Original (*C*).	Treated (*E*).		pH	Refractive index.	pH	Refractive index.
		mos.							
1	32142	3	3.68	2.91		8.0	1.3338	8.0	1.3322
2	32153	3	3.83	3.08	0.80	8.0	1.3338	8.0	1.3322
3	31719	10	4.96	4.55		8.2	1.3370	8.2	1.3343
4	31732	10	3.59	3.11	0.90	8.2	1.3370	8.2	1.3343
5	31782	10	3.35	2.64		7.2	1.3410	7.2	1.3386
6	31798	10	3.03	2.43	0.81	7.2	1.3410	7.2	1.3386
		yr.							
7	32101	1	2.22	1.85		7.8	1.3360	7.8	1.3348
8	32112	1	3.28	2.84	0.87	7.8	1.3360	7.8	1.3348
Average.....					0.83				

* Each group averaged from three to four experiments.

TABLE VIII.

Action of Serum, after Removal of CO_2 Precipitate, from Old Chickens, on the Rate of Growth of Homologous Fibroblasts.

Group No.*	Culture No.	Age of animal.	Rate of growth in serum.		Ratio: $\frac{E}{C}$.	Serum.			
						Original.		Treated.	
			Original (*C*).	Treated (*E*).		pH	Refractive index.	pH	Refractive index.
		yrs.							
1	32122	3	2.23	2.09	0.94	7.8	1.3408	7.8	1.3372
2	32100	6	1.84	1.71		7.8	1.3361	7.8	1.3350
3	32111	6	2.23	2.09	0.93	7.8	1.3361	7.8	1.3350
Average.....					0.94				

*Each group was composed of twelve experiments.

IV.

Action on Homologous Fibroblasts of the Serum of Young and Old Animals after the Removal of the Carbon Dioxide Precipitate.

In twenty-eight experiments, divided into eight groups, the effects of the residual sera of the young animals were compared to those of the respective normal sera. It was found that the residual serum had become 17 per cent more inhibiting than the normal serum (Table VII). In three groups of twelve experiments, the action of the residual sera of the old animals was compared to that of the respective normal sera. There was practically no difference in the action of the normal and residual sera within the limits of experimental error (Table VIII).

V.

CONCLUSIONS.

It may be concluded that, under the conditions of the experiments:

1. The inhibiting action on homologous fibroblasts of the heated serum of a young animal increases relatively more than that of an old animal. After it has been heated, the inhibiting action of the serum of the old animal is still greater than that of the young animal.

2. The CO_2 precipitate obtained from the serum of a young animal definitely increases the proliferative activity of homologous fibroblasts, while the CO_2 precipitate of the serum of an old animal has practically no activating power. After the removal of the CO_2 precipitate, the inhibiting action of the serum of young animals is increased, while that of old animals is not modified.

3. The increased inhibiting action of serum on homologous fibroblasts in old age is partly due to the disappearance of the growth-activating substances and to the enhanced activity of the growth-inhibiting principle.

STUDIES ON THE NASOPHARYNGEAL SECRETIONS FROM PATIENTS WITH COMMON COLDS.

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(Received for publication, April 18, 1923.)

The experimental studies on the incitant of common colds in man to be reported in this paper were begun shortly after the investigations on influenza by Olitsky and Gates [1–3] were undertaken. They were initiated by the desire to check or control the latter studies and to determine, if possible, any causative agent in the nasopharyngeal secretions derived from persons suffering from common colds. This work has now extended over 4 years, and, while still incomplete, our purpose is to state only the results which we regard as established experimentally.

Source of Materials.

It is obvious that patients subjected to study should be selected carefully, for the pathognomonic symptom, rhinitis, can be induced by exposure to cold and by other physical and chemical agents, and is a sign of several affections, such as hay-fever, influenza, measles, and other exanthemata. Only such affected individuals were chosen who could trace their illness to exposure to a previous case of common cold within 3 or 4 days; in other words, only those who had an infectious condition and showed at the same time the typical syndrome of the disease. This consists of premonitory malaise and dryness of the nasal passages, followed by sneezing and discharge of a profuse, thin, irritating secretion from the nose. Conjunctivitis of a mild degree with

[1] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 125, 361, 373, 713; xxxiv, 1; 1922, xxxv, 1, 553, 813.

[2] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1922, xxxvi, 501.

[3] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1922, xxxvi, 685; 1923, xxxvii, 303; *Science*, 1923, lvii, 159.

lacrimation is usually present and in some persons slight headache. The temperature is either not elevated or only slightly so and in general the constitutional reaction is negligible. On the 2nd or 3rd day the nasal discharge diminishes in quantity, becomes first mucoid and then purulent; the mild constitutional symptoms, if present at the beginning, disappear, and in uncomplicated cases, complete recovery ensues after 4 or 5 days. In the purulent stage, however, secondary localizations may occur by extension of the inflammation to the ear, inducing otitis media, or to the paranasal sinuses, or to the larynx. In these instances, the microorganisms usually found associated are the staphylococcus, streptococcus, pneumococcus, and Friedländer bacillus groups, the meningococcus, *Micrococcus catarrhalis*, and Pfeiffer's bacillus.[4,5]

Ringer's or 0.85 per cent saline solutions were employed to wash the nasopharyngeal secretions from cases of common colds. These washings were collected from 40 patients in the early hours (from 3 to 20) of the uncomplicated illness. In addition, washings were obtained from 30 individuals, supposedly free from common colds, and were employed as controls. In general the methods used in the following experiments were those described in the series of papers on influenza by Olitsky and Gates.[1–3]

Transmission Experiments.

Common cold is a mild disease in man; constitutional reaction is either lacking or is transitory and inconsequential. There is no definite, measurable criterion of a positive transmission to rabbits—the animals employed in the experiments. The symptoms of nasal discharge and sneezing cannot be regarded as critical in rabbits, for these animals, as a future report will show, develop snuffles spontaneously in association at times with an underlying chronic paranasal sinusitis. Thus, after nasal swabbing of thirteen rabbits with filtered and unfiltered nasopharyngeal washings from fresh cases of common cold in man, six showed, within 2 to 4 days thereafter, symptoms of nasal

[4] Lacy, G. R., *J. Lab. and Clin. Med.*, 1918, iv, 55.

[5] Crowe, S. J., and Thacker-Neville, W. S., *Bull. Johns. Hopkins Hosp.*, 1919, xxx, 322.

discharge, a proportion which prevailed, however, among an equal number of animals swabbed with control materials. Similarly, the intratracheal injection into fifteen rabbits of the unfiltered nasopharyngeal washings derived from ten cases of early common colds was followed by no constant or characteristic effects. Although the lung tissue of injected rabbits was reinoculated into two successive series of animals, the results of these intratracheal inoculations showed that some animals gave a polynucleosis with frank lobar pneumonia, from the lungs of which either the pneumococcus or streptococcus or rabbit septicemia bacillus could be recovered in pure culture, others a mononucleosis without lung involvement, and the remainder, inconstant effects. Similar results were obtained from the washings of six supposedly normal individuals injected intratracheally into eleven rabbits.

The conclusions to be drawn from our attempts at transmission to rabbits are that, in the absence of a suitable criterion for a positive transmission or because of the mildness of the disease, these animals failed to show any definite effects. On the other hand, it is apparent that experimentally the nasopharyngeal secretions of early cases of common colds are distinct from those of influenza, since the latter, as already demonstrated,[1–3] induce constant and striking effects both in the blood and on the lungs of rabbits.

Transmission to Man.

It is apparent that a disease of so mild and transitory a nature as the common cold could best be studied, in as far as transmission of any possible incitant is concerned, in man. Before describing the experiments the previous work of Kruse,[6] in 1914, and Foster,[7] in 1915–16, should be mentioned.

Kruse diluted the nasal secretion of an assistant ill with a common cold with 15 times its volume of saline solution and then filtered the material through a Berkefeld candle. A few drops of the filtrate were instilled into the nose of each of twelve men. In 1 to 3 days, four of them showed common colds. On repetition, the nasal discharge from a case of common cold was taken up in 20 volumes of saline solution and instilled into the nose of each of 36 volunteers. In 1 to 4 days

[6] Kruse, W., *Münch. med. Woch.*, 1914, lxi, 1547.

[7] Foster, G. B., Jr., *J. Infect. Dis.*, 1917, xxi, 451; *J. Am. Med. Assn.*, 1916, lxvi, 1180.

fifteen, or 42 per cent, became ill with this disease. Kruse concluded that the incitant of common colds is a filterable microorganism, although he was unable to demonstrate either morphologically or by culture a formed, living and multiplying element.

Foster later believed that he confirmed the findings of Kruse. The nasal secretions from cases of common colds within 24 hours after the onset of the first symptoms were suspended in 10 cc. of saline solution and, after shaking, the homogeneous suspension was filtered through a Berkefeld N candle. From 3 to 6 drops of the filtrate were instilled into both sides of the nose in each of ten men. "7 developed clear cut and definite symptoms of acute coryza; 2 reacted questionably, while 1 remaining case exhibited no symptoms. There was an incubation period of 6 or 8–30 hours. . . . . The duration of the symptoms varied 3–6 days—usually 5." In addition, Foster stated that he has cultivated in fresh tissue—ascitic fluid medium from eleven cases of the natural disease and from five of experimental common colds a minute anaerobic microorganism similar in many respects to the "globoid bodies" of Flexner and Noguchi.[8]

The present experiments were made on nineteen volunteers with the washings collected from eight patients. The mode of procedure differed somewhat from that of Kruse and Foster. Instead of the nasal discharges, we obtained the nasopharyngeal secretions by washing with 25 to 30 cc. of either Ringer's or 0.85 per cent saline solutions.[1–3] The suspension was shaken and then filtered through a Berkefeld V or N candle. The clear filtrate free from ordinary bacteria, as determined by cultivation tests, was applied to cotton applicators 2 cm. long and 0.5 cm. wide, the cotton being thoroughly soaked, and then conveyed to the nasal mucous membrane, on each side, by gentle swabbing. In each series of experiments one or two volunteers were swabbed with the unheated filtrate and another, as a control, with the same material but heated at 60°C. for 1 hour. Furthermore, the cases were earlier in the course of the illness than those reported by Foster; namely, from 3 to 20 hours after the onset of the first symptoms.

Experiments on transmission from man to man were attempted with the filtered nasopharyngeal secretions from six patients in the early stages of infectious common colds. The following protocols are illustrative of the tests.

[8] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

Protocol 1. Patient A.—Exposed to a case of a common cold 3 days previous to onset of symptoms. Illness characterized by a profuse, serous, irritating discharge from nose, slight conjunctivitis, but practically no fever or constitutional symptoms. This lasted for 2 days, after which the patient returned to normal. The nasopharyngeal secretions were collected in 25 cc. of Ringer's solution, 3 hours after the onset of the first signs of the affection. The unfiltered washings on aerobic culture showed growths of *Staphylococcus albus* and Pfeiffer's bacillus. The filtered washings on aerobic blood agar plate cultures yielded no growth. Anaerobic cultures of this and the following patients to be mentioned will be described later.

The filtrate was separated into two portions: one, unheated, was applied by swabbing to the nasal mucosa of Volunteer 1; the other, heated at 60°C. for 1 hour, to that of Volunteer 2. Volunteer 2 was unaffected.

Volunteer 1, in the afternoon of the 2nd day after swabbing, complained of malaise and dryness of the nose. The next morning he presented a profuse, thin serous discharge from the nose, felt feverish, and suffered from headache. The following day the symptoms were aggravated, and the nasal discharge, which was profuse and mucoid, became purulent on the 4th day of the illness. During the 5th to 7th days the affection subsided; the constitutional symptoms disappeared and only a slight purulent discharge was noted; but on the 8th day a right maxillary antrum infection supervened which endured for 2 weeks.

18 hours after the onset of the first symptom, the nasopharyngeal secretions of this person were collected in 25 cc. of Ringer's solution. The unfiltered washings showed on aerobic culture an occasional colony of *Staphylococcus albus* and *Streptococcus viridans;* the filtered washings yielded no growth.

The filtrate was treated as in the case of Patient A and applied, unheated, to the nasal mucosa of Volunteer 3 and heated on that of Volunteer 4. The latter was unaffected. Volunteer 3, who is ordinarily resistant to common colds, showed after 24 hours a profuse, thin discharge from the nose without any other symptoms. This lasted for only 1 day, after which the discharge disappeared.

Protocol 2. Patient B.—Exposed to a case of a common cold 2 days previous to onset of symptoms. There were no constitutional symptoms in this individual, only a profuse serous and later mucoid discharge from the nose and lacrimation, which endured for 2 days.

12 hours after the onset, the nasopharyngeal secretions were collected in 25 cc. of Ringer's solution. The unfiltered secretions yielded an occasional colony of *Staphylococcus albus*, Pfeiffer's bacillus, and diphtheroids on aerobic culture; the filtered material exhibited no growth.

Volunteer 5 was swabbed nasally with the unheated filtrate, Volunteer 6 with the heated. The latter was not affected. Volunteer 5, 8 hours after swabbing, felt an increase of mucus in the nasopharynx. On the next day there was a profuse, thin, serous secretion from the nasal mucosa but without constitutional disturbance. On the 2nd day the discharge became mucoid and the nose was clogged so that he was forced to breathe through the mouth. On the 3rd day the

nasal secretions were profuse and purulent. The following day saw improvement in his condition, but on the 5th day there was an exacerbation of the acute attack. The duration of illness was 6 to 7 days.

20 hours after the onset of the affection the nasopharyngeal secretions of Volunteer 5 were collected in 30 cc. of Ringer's solution. The aerobic cultures of the unfiltered washings gave a few colonies of *Micrococcus flavus* and *Streptococcus viridans;* those of the filtrate were sterile.

The heated filtrate was swabbed on the nasal mucosa of Volunteer 7, who was not affected, and the unheated filtrate on that of Volunteer 8, who exhibited 8 hours later dryness of the nasal mucous membrane; 17 hours later there was an increased thin discharge without lacrimation or constitutional disturbance. 24 to 48 hours later the discharge was thicker, mucoid, and profuse, and on the 3rd day the subject was well.

Protocol 3. Patient C.—Two associates of this woman, including herself, became ill with common colds within 2 to 3 days of each other. 5 days after the onset of symptoms, her mother became ill with this disease. Patient C suffered from a mild attack; the 1st day was characterized by a profuse serous nasal discharge, the 2nd by a change in the nature of the secretions to mucoid and the 3rd day to mucopurulent. Except for lacrimation there were no symptoms.

6 hours after the onset, the nasopharyngeal secretions were washed with 30 cc. of Ringer's solution. The unfiltered washings yielded on aerobic culture numerous colonies of *Staphylococcus albus;* the filtered, no growth.

The filtrate was swabbed on the nasal mucosa of Volunteer 9. 14 hours later sneezing and dryness of nasal mucous membrane were noted. The next day this person showed injected conjunctivæ with lacrimation, sneezing and dryness of nasal mucosa, slight headache, and malaise. The following day there was a thin, serous discharge from the nose and slight laryngitis. The nasal symptoms and the laryngitis lasted for a day; thereafter the subject gradually returned to normal.

The nasopharyngeal washings from this volunteer were obtained 48 hours after the onset. The unfiltered material showed on aerobic culture a few colonies of *Micrococcus flavus* and *Streptococcus viridans;* similar tests on the filtrate were negative. The filtrate was swabbed on the nasal mucosa of Volunteer 10 who was not affected.

Protocol 4. Patient D.—This patient was in contact with two cases of common colds, one a week, the other 3 days previously. He had a profuse, thin, irritating, nasal discharge with excoriated skin about the nose. This condition endured for 3 days.

18 hours after the onset of symptoms, the nasopharyngeal secretions were collected by washing with 25 cc. of Ringer's solution. The unfiltered washings yielded a pure culture of aerobic diphtheroid bacilli; the filtered material was free from aerobic bacteria.

The heated filtrate was applied to the nasal mucosa of Volunteer 11, who remained normal. The unheated filtrate was swabbed on the nasal mucous membrane of Volunteer 12, who 24 hours later exhibited a profuse, mucoid, nasal dis-

charge with collection of mucus in the nasopharynx. 48 hours later this person had a profuse, mucopurulent, nasal discharge and perspired freely (a symptom ordinarily concomitant with common colds in this man's experience). The symptoms disappeared 2 days later.

No attempts at further transmission were made in this instance.

Protocol 5. Patient E.—Contact with common cold case 2 days previous to onset of symptoms which were typical of those of a common cold. The nasopharynx was washed with 25 cc. of Ringer's solution 18 hours in the course of the affection. The unfiltered washings yielded Pfeiffer's bacillus, *Staphylococcus aureus*, and *Streptococcus viridans;* the filtered washings were free from aerobic bacteria.

The heated filtrate was swabbed on the nasal mucosa of Volunteer 12 and the unheated on that of Volunteers 13 and 14. None of these persons was affected.

Protocol 6. Patient F.—This patient was exposed to three others with common colds 3 days before the onset of his illness. The attack was typical and endured for 4 days. 20 hours after the onset of symptoms, the nasopharyngeal secretions were collected in 30 cc. of Ringer's solution. The unfiltered washings exhibited a few colonies of *Staphylococcus albus* and *Streptococcus viridans* on aerobic plate cultures; the filtered washings were free from growth.

The heated filtrate was applied to the nasal mucosa of Volunteer 15 and the unheated to that of Volunteers 16 and 17. All remained normal.

The experiments, although limited in number indicate the transmission of a condition similar to common colds from man to man with the filtered nasopharyngeal washings derived from four of six patients in the early hours of the disease. The two patients with whom transmission failed were 18 and 20 hours in the course of a common cold; the four from whom positive results were obtained were ill for 3, 6, 12, and 18 hours when the tests were made. In two of these four cases, transmission was effected to a second person; in one, such transmission failed, and in the fourth no attempt was made beyond the first passage. Altogether, therefore, definite symptoms were induced in six persons, of whom one developed a secondary maxillary antrum infection and the other a secondary laryngitis. Of the six persons, the first symptoms were noted 8 hours after swabbing in two cases, 24 hours in two, and 14 and 48 hours in the fifth and sixth individuals.

Control Experiments.

In the foregoing experiments it will be noted that the filtrates of the nasopharyngeal washings from early cases of common colds,

heated for 1 hour at 60°C., failed to produce any effects in seven individuals. It seemed, however, that if parallel experiments should be made with the filtrates derived from cases regarded as suffering from non-infectious "colds," the evidence to be deduced from the control series would be strengthened.

Protocol 7. Patient G.—There was no contact with a case of a common cold but a definite history of exposure to cold and wet weather. Since then the patient had had a running nose without other manifestations, which lasted for 2 days.

12 hours after the onset, the nasopharyngeal secretions were washed with 25 cc. of Ringer's solution. The washings, which showed on aerobic culture *Streptococcus viridans* and Pfeiffer's bacillus, were filtered. The filtrate, free from aerobic bacteria, was swabbed on the nasal mucosa of Volunteer 18, who remained unaffected.

Protocol 8. Patient H.—There was no contact with a case of a common cold. Patient was exposed to continuous cold weather in an all day automobile ride. A thin discharge from the nose occurred on the next day and continued until the following noon. The nasopharyngeal secretions were collected in the first 3 to 4 hours after the onset in 25 cc. of Ringer's solution. Aerobic plate cultures yielded five colonies of *Staphylococcus aureus* from the unfiltered washings but no growth from the filtered material. The filtrate was swabbed on the nasal mucous membrane of Volunteer 19, who was not affected.

These two persons who evidently had non-infectious colds did not induce the transmission effects in inoculated individuals.

Cultivation Experiments.

We have already indicated, in the foregoing protocols, that cultures by ordinary means, such as by the employment of rabbit blood agar plates or veal infusion broth under aerobic conditions, did not yield growths of any constant or distinctively pathogenic microorganisms from either the nasopharyngeal washings or from rabbit lungs inoculated with these materials. The results with anaerobic methods will now be described.

The filtered nasopharyngeal secretions of 40 patients with early, typical, infectious common colds, and the unfiltered lung tissue suspensions of fifteen rabbits injected intratracheally with the secretions from ten of the patients, were cultured in the Smith-Noguchi, fresh tissue-ascitic fluid medium under a petrolatum seal. In none of the cultures was any predominating, pathogenic microorganism seen. It

is noteworthy that in no instance was *Bacterium pneumosintes* found. In this regard, the nasopharyngeal secretions derived from common colds differ from those obtained in influenza, since this bacterium has been shown to bear a definite relationship to influenza.[1-3] We were also unable to cultivate the so-called "globoid bodies" of Foster.[7] The appearance of precipitate in this medium simulating morphologically definite bodies will be discussed below.

Later in the course of the cultivation experiments the anaerobic plate method was also used. This has already been described[1-3] and consists of 5 per cent rabbit blood plain agar in ordinary Petri dishes placed in the Brown anaerobic jar.[9] The advantages of the anaerobic plates in conjunction with the Smith-Noguchi fluid medium have been demonstrated in connection with the cultivation experiments with influenzal materials.[2]

The combined method of Smith-Noguchi medium and the anaerobic plates was employed in the cultivation of the filtered nasopharyngeal washings from nineteen of the forty cases mentioned. This procedure also failed to reveal a constant, definite, pathogenic agent, or Foster's globoid bodies. By means of the anaerobic plates, however, a number of microorganisms were isolated belonging to the three groups of anaerobic, filter-passing, Gram-negative bacteria described by Olitsky and Gates.[2]

Representatives of the first group of these microorganisms which are actively motile, spirochete-like, slender, curved bacilli, were obtained in eight of the nineteen cases studied. In two of the eight, bacteria of the other two groups were also found; in one, those of the second group and in the other case those of the third group. In a parallel series of experiments with six supposedly healthy individuals and six patients in the early hours of epidemic influenza, similar microorganisms were isolated in three of the normal controls and in two of the influenza cases. Of the latter two, in one instance bacteria of the third group were also obtained. In addition, this type of organism was recovered from a patient suffering from follicular tonsillitis.

The second group consists of minute, tenuous, pleomorphic bacilli. A representative of this group was obtained in one of the nineteen

[9] Brown, J. H., *J. Exp. Med.*, 1921, xxxiii, 677; 1922, xxxv, 467.

cases of common colds in the culture of which bacteria of Group I were also noted. The same species was cultivated from the nasopharyngeal secretions of one of two cases of acute follicular tonsillitis but not from those of nine supposedly healthy subjects or from the six cases of influenza.

The third group comprises bacteria of actively motile, very tenuous, slender, vibrio-like microorganisms which are smaller than those of the first group and are characterized by their failure to grow in fluid medium. Bacteria of this class were found in three of the nineteen cases of common colds, and in one of the three were associated with microorganisms of the first group. The same species was isolated in four of nine supposedly healthy individuals and in one of six cases of influenza.

Additional serological tests to those already reported[2] indicate that the rabbit immune serum produced by repeated injections of each type of these microorganisms shows each group to be distinct from the others; there is no cross-agglutination among the different types. Furthermore, these rabbit immune sera fail to agglutinate *Bacterium pneumosintes* and conversely, anti-*pneumosintes* immune serum does not agglutinate any of the microorganisms in the three groups.

Live cultures of fourteen strains of these "group" bacteria were injected intratracheally in rabbits to test their pathogenicity. None of the rabbits exhibited distinctive changes either in the blood or lungs or temperature. In only two instances were bacteria recovered from the lungs of the rabbits similar to those inoculated. Both of these microorganisms belonged to the first group. In addition to the rabbit tests for pathogenicity, a culture of each type was swabbed on the nasal mucosa of each of three persons. None was affected.

To sum up the cultivation experiments with the filtered nasopharyngeal secretions from early cases of typical infectious common colds, it appears that no single, constant, pathogenic agent was found in aerobic or anaerobic media. By means of a combination of Smith-Noguchi medium and anaerobic rabbit blood agar plates, however, a number of microorganisms belonging to the three groups already described by Olitsky and Gates[2] were isolated. Their irregularity of occurrence in common colds, influenza, and supposedly normal individuals, their differentiation into three distinct species, and their lack of

pathogenicity for rabbits or man would indicate that they are not the incitants of common colds. Finally, it is to be emphasized that *Bacterium pneumosintes* was not found in any of the cultures from the nasopharyngeal secretions of common colds nor from those of the supposedly normal persons employed as controls.

DISCUSSION AND SUMMARY.

From the foregoing experiments it appears that with the filtered nasopharyngeal secretions from early cases of typical infectious common colds in the first 3 to 18 hours of the disease, a similar condition can be transmitted to man. With the unheated but not with the heated secretions from four of six such patients we have succeeded in transmitting an affection indistinguishable from common cold to four men and in two instances the condition was conveyed from the person with the experimental disease to a second individual—in all, therefore, to six supposedly normal subjects. The periods of incubation in the experimental disease varied from 8 to 48 hours. We failed to obtain these results with the filtered secretions from cases of common colds 18 and 20 hours after the onset of symptoms and from a patient with the experimental disease 20 hours after the first symptoms. It would appear that the secretions are more active in the early hours of the affection. We also failed in the two instances in which colds were caused by exposure to the elements, or chilling of the body, and not by definite contact with other cases of common colds.

Intratracheal inoculations in rabbits with unfiltered and filtered nasopharyngeal washings obtained from patients with common colds induce no characteristic or distinctive effects on the tissues, from which no constant, pathogenic agent has, as yet, been recovered. In comparison, similar material from cases of epidemic influenza do, however, cause particular changes in the blood and lungs of these animals, cultures of the lungs often yielding *Bacterium pneumosintes*.[1-3] In view of these facts and since the clinical pictures exhibited by these diseases differ, the conclusion may be drawn that infectious common colds and epidemic influenza are separate and distinct diseases. On the other hand, the negative results obtained with materials derived from common colds and from parallel series of experiments with secretions

from supposedly healthy persons, serve as a control to the effects produced with the nasopharyngeal washings obtained from influenza patients.[1–3]

Aerobic and anaerobic cultures of the filtered nasopharyngeal washings from 40 early cases of infectious common colds have thus far yielded no constant, pathogenic agent which can be regarded as the incitant of the disease. The filtered washings of nineteen cases were studied by the combined method of Smith-Noguchi fluid medium and anaerobic blood agar plates. In these instances representatives of the three groups of anaerobic filter-passing, Gram-negative bacteria, described by Olitsky and Gates[2] were cultured from twelve patients. The irregularity of their occurrence not only in common colds but in influenza and supposedly normal persons and their lack of pathogenicity for rabbits and man indicate that these bacteria are not peculiar to common colds. This method has opened to view a number of hitherto undescribed microorganisms which can be found in different respiratory affections and in health. Furthermore, by morphological, cultural, and serological means, the separation into distinct species of each of these groups of bacteria has again been demonstrated. It is noteworthy that *Bacterium pneumosintes* was not found in any of the cultures from the 40 patients.

Special attention was given to the detection of elements similar to Foster's globoid bodies in the cultures derived from common colds and from the experimental disease in man, and from the lungs of inoculated rabbits. We have not been able to determine the presence of these bodies, although the precipitate which forms in fresh rabbit kidney tissue-ascitic fluid medium was illusory in such relation since it was a common experience to find this precipitate simulating the globoid bodies of poliomyelitis. Still more disturbing is the fact, that these particles could be carried over from subplant to subplant and even showed pseudo colony formation in the Noguchi semisolid medium in tubes.[3] But when the particles were put to rigorous test for a living and multiplying organism, the tests failed to reveal multiplication.

The increasing importance of the tissue-ascitic fluid medium in bacteriological technique warrants a detailed description of the requirements necessary for the determination of the living nature of formed

elements in cultures in this medium. (*a*) No one method of staining can be relied upon, for stain precipitate in itself adds to the confusion. A specimen for examination should be stained separately by Gram's and Giemsa's methods and with another nuclear dye, such as polychrome methylene blue. As a rule, microorganisms will reveal their morphological characters in more than one stain, whereas precipitate may be found in only one and not in the others. The experienced eye will discern the precipitated particles in selected parts of the stained preparation where they often occur in enormous numbers, clumped into irregular masses of varying forms from the periphery of which there is a gradual fading out to finer, more uniform structures. (*b*) In addition, suspected growths should be tested in the dialysate medium of Gates[10] since by this method the precipitated material of the medium is kept from admixing with the growth, and a clear view of any microorganisms, if present, is obtained. (*c*) Another requirement is colony formation of the suspected culture. This is an absolute essential and can be effected by planting the material to be tested on solid plate media, incubated aerobically, and anaerobically in Brown's[9] jar. Semisolid medium in long tubes should also be employed but care is needed to avoid mistaking small particles of precipitate for actual colonies of bacteria. To make certain of growth of microorganisms in semisolid medium, however, subplanting to a precipitate-free, dialysate medium is required.

By following this method minute microorganisms which are obscured, or simulated, by precipitate in the Smith-Noguchi medium, can be identified.

CONCLUSIONS.

The transmission of a clinical condition similar to typical, infectious common cold from man to man with the filtered nasopharyngeal washings of early cases of the disease indicates that the incitant is filterable, thus confirming the earlier observations of Kruse and Foster.

[10] Gates, F. L., *J. Exp. Med.*, 1922, xxxv, 635.

Experiments on rabbits with these secretions and cultivation tests show that the materials derived from patients with common colds are distinct in effects from those of epidemic influenza.

Cultivations of the nasopharyngeal washings from 40 cases, and from the lung tissue of inoculated rabbits, have failed to reveal any constant, pathogenic agent, or incitant. Although a careful search was made for the "globoid bodies" of Foster in these materials, we have been unable to find them. None of these cultures, furthermore, yielded *Bacterium pneumosintes*.

A METHOD FOR THE DETERMINATION OF THE SPECIFIC GRAVITY OF RED BLOOD CELLS.

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(Received for publication, March 9, 1923.)

In determining the specific gravity of red blood cells, the corpuscles alone should be studied. It is obvious that no direct information can be obtained from an investigation of whole blood, as the specific gravity of plasma is different from that of the cells. Two general methods for ascertaining the specific gravity of the corpuscles are available. One consists in weighing a known volume of red blood cells under constant conditions. Such a procedure, with cells completely freed from plasma, involves careful analytical work and is time-consuming. The second method is more simple and depends upon the use of two miscible liquids of different specific gravities, into which the sedimented corpuscles can be dropped. By varying the proportions of the liquids, a medium can be obtained in which the cells neither sink nor rise but remain suspended. The specific gravity of the red blood cells is the same as that of the mixture.

A suitable mixture must fulfill the following requirements. (*a*) The two fluids must be completely miscible in all proportions. (*b*) One must have a greater and one a lower specific gravity than red blood cells. (*c*) Neither individually, nor mixed, must these liquids dissolve any of the constituents of the red blood cells, or mix with them or enter into any chemical or physical reaction with the corpuscles. In other words, the liquids must be inert with respect to blood.

Roy (1) used mixtures of water and glycerol. Neither is fitted for specific gravity determinations because both extract the constituents of cells. Hammerschlag (2) suggested benzene and chloroform. Both of these dissolve lecithin and cholesterol from corpuscles. If whole blood is added to mixtures of chloroform and benzene, the plasma protects the red blood cells. The protection, however,

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lasts but a short time and the cells rapidly change in specific gravity (3). If red blood cells, freed from plasma, are used, they immediately become distorted in chloroform-benzene mixtures and soon hemolyze.

In searching for fluids which fulfill the requirements previously mentioned, benzyl benzoate was found to be admirably suited for the heavy liquid. It has a specific gravity of 1.115 at 20°C. and when mixed with sedimented corpuscles does not dissolve lecithin or cholesterol from them. If crystals of cholesterol are mixed with benzyl benzoate, they dissolve to some extent, but no acetic anhydride reaction is obtained when red blood cells are used. Chloroform, in which corpuscles have been suspended, gives a marked cholesterol reaction. A drop of sedimented cells maintains its shape in benzyl benzoate and shows no hemolysis in 24 hours. If the red blood cells are placed on a slide with a drop of benzyl benzoate and the cover-slip is sealed by vaseline, no microscopic change is evident in 24 hours.

For the lighter fluid, cottonseed oil was found to be most suitable. It is miscible with benzyl benzoate in all proportions. Neither lecithin nor cholesterol is soluble in it. No microscopic or macroscopic changes in the red blood cells are apparent when mixed with cottonseed oil. The oil used in this work had a specific gravity at 20°C. of 0.920.

If mixtures of benzyl benzoate and cottonseed oil are made up, varying from five parts each to ten parts of the heavy liquid and none of the oil, a series is obtained with specific gravities ranging from 1.017 to 1.115. A set of small Wassermann tubes containing 2 cc. of each mixture is prepared for each determination. A variation of 0.02 cc. of either fluid changes the specific gravity by 0.002.

The red cells are prepared by centrifuging defibrinated blood in small Wassermann tubes for 15 minutes at 3,600 revolutions per minute. The supernatant plasma is entirely removed by means of a nipple-capped pipette. The sediment appears homogeneously red under the microscope, no plasma being observed, and the supernatant fluid appears to be no more tinged by hemoglobin than the plasma resulting from standing. In other words, the cells appear to be intact after the vigorous centrifugalization. A drop from this thick sediment is used for the test.

Table I shows comparative results obtained with five specimens by the benzyl benzoate-oil method and pycnometer determinations. The latter were carried out in the usual manner. The pycnometer, calibrated with distilled water, was filled with whole blood and placed in the thermostat at 25°C. for 30 minutes. After drawing the blood to the mark, the pycnometer was dried and weighed. The same procedure was carried out with plasma. With the aid of hematocrit determinations the specific gravity of the red blood cells was then calculated as follows:

x = specific gravity of red blood cells.

$$\begin{pmatrix}\text{Per cent of red}\\ \text{blood cells in}\\ \text{whole blood}\end{pmatrix} x = \text{specific gravity of whole blood} - \begin{pmatrix}\text{Per cent of}\\ \text{plasma in}\\ \text{whole blood}\end{pmatrix}\begin{pmatrix}\text{Specific}\\ \text{gravity}\\ \text{of plasma}\end{pmatrix}$$

TABLE I.

Blood specimen.	Weight of whole blood.	Weight of plasma.	Specific gravity of whole blood.	Specific gravity of plasma.	Hematocrit determination. Per cent red blood cells.	Specific gravity of red blood cells calculated.	Specific gravity of red blood cells by benzyl benzoate-oil method.
	gm.	*gm.*					
1	1.3279	1.2838	1.0618	1.0269	52	1.0924	1.092
2	1.3126	1.2813	1.0498	1.0249	36	1.0941	1.093
3	1.3173	1.2806	1.0537	1.0244	41.4	1.095	1.094
4	1.3205	1.2819	1.0563	1.0254	45	1.0941	1.094
5	1.3188	1.2804	1.0549	1.0242	44.5	1.0931	1.092

The table shows that in every instance the agreement is within 0.1 per cent. This is very close when it is considered that the error in hematocrit determinations is often as high as 1.0 per cent.

Table II shows results obtained in a few routine tests. These results show the variations in a few cases. It is to be noted that the washed red blood cells have a higher specific gravity than corpuscles centrifuged from whole blood. This change is probably due to the obvious diffusions from the red blood cells when washed. Corpuscles in 0.6 per cent saline concentration swell, and in 1.3 per cent shrink. The passage of water is naturally accompanied by a change in specific gravity as demonstrated in the table.

When several properties of the blood are under investigation, it is desirable to use rapid and accurate methods. Opitz and Frei (4)

recently studied the relation between the specific gravity and sedimentation rate of the red blood cells, using the pycnometer method. For just such experiments is the benzyl benzoate-oil method especially fitted by its accuracy, rapidity, and simplicity. Several determinations can be carried out in one-fourth the time required to do one by the pycnometer.

TABLE II.

Blood specimen.	Specific gravity.				
	Red blood cells from defibrinated whole blood.	Red blood cells washed free from plasma with Ringer's solution.			Red blood cells washed free from plasma with Ringer's solution standing at 30°C. for varying intervals of time.
		In 0.9 per cent Ringer's solution.	In 0.5 per cent Ringer's solution.	In 1.3 per cent Ringer's solution.	
1	1.093	1.097			6 hrs., 1.097.
2	1.096				
3					½ hr., 1.090; 1 hr., 1.085; 2 hrs., 1.088; 3 hrs., 1.090; 4 hrs., 1.091; 5 hrs., 1.094; 6 hrs., 1.087.
4		1.096	1.069	1.113	
5		1.089	1.065	1.109	
6		1.092			
		1.090			
7	1.089	1.096	1.063	1.108	9 hrs., 1.091.
			1.061	1.107	
8	1.081	1.087	1.069	1.104	3 hrs., 1.089; 10½ hrs., 1.084.
			1.073	1.107	

CONCLUSIONS.

1. A simple method for determining the specific gravity of red blood cells is described.

2. It is more rapid than and as accurate as pycnometer determinations.

3. The marked chemical and physical interactions between corpuscles and suspension fluids in other methods are avoided.

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THE LUNG VOLUME IN HEART DISEASE.

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(Received for publication, March 19, 1923.)

INTRODUCTION.

In heart failure it is assumed that the supply of O_2 to the tissues and the removal of CO_2 from them is not commensurate with the needs of the organism. Pearce (1) has emphasized the intimate relationship existing between respiration, circulation, and metabolism. No longer can any of these functions be regarded as isolated, but they must be considered together as a physiological system. The reason for studying lung volume in heart disease is that it aids in an understanding of the interdependence of cardiac and respiratory failure.

Bohr (2) and Hasselbalch (3) have emphasized the importance of the simultaneous determination of the total lung volume and its subdivisions. In an attempt to visualize the degree of pulmonary distension and the relations between air content in the lung and blood flow in the lesser circulation a knowledge of the vital capacity alone may lead to error. The study of the vital capacity of the lungs in heart disease has become of great practical interest, owing largely to the work of Peabody and his associates (4). Study of the vital capacity alone, however, can throw little further light on the pathologic changes, both structural and functional, which give rise to the phenomenon of dyspnea.

Since Bohr reawakened interest in pulmometry, numerous investigators (Rubow (5), Bruns (6), Bittorf and Forschbach (7), and Siebeck (8)) have studied the total air capacity of the lungs (vital capacity and residual air) in cases of heart disease. These investigators used the gas mixture method described by Bohr (2) for the determination of the volume of residual air. Two of these investigators (Bruns (6) and Siebeck(8)), dwell with considerable emphasis on the difficulty of applying Bohr's method to patients with heart disease in whose lungs normal gaseous diffusion, they believe, is disturbed. In this country, Peters (9), using the method of Lundsgaard and Van Slyke (10), found the decrease in vital capacity in heart disease to be accompanied by a decrease in total lung capacity and residual air. Lundsgaard's (11) recent important contributions to the subject will be discussed

later. In our hands the procedure of mixing used by Lundsgaard and Van Slyke (10) has not proved applicable to the study of those patients with heart disease in whom any considerable degree of dyspnea is present. This fact has been discussed in another communication (12) in which a new method for measuring lung volume was described. The present paper deals with the results obtained by applying this method in the study of a series of cases of heart disease.

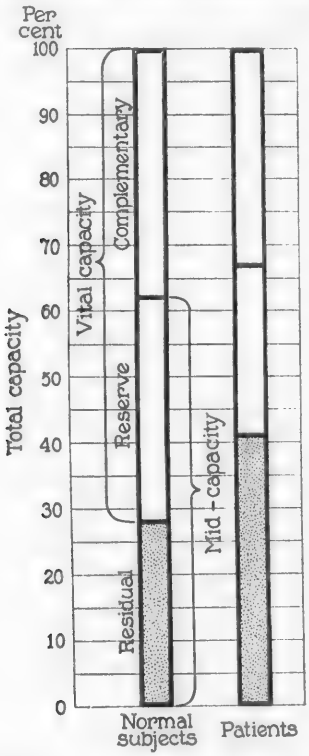
Material.—The material for study was selected from a group of cases suffering from various types of chronic heart disease.

Most of the patients had disturbances of the cardiac mechanism as evidenced by the presence of auricular fibrillation. The advantage of studying patients with fibrillation of the auricles lies in the fact that the heart rate and usually the state of decompensation in these patients can be controlled by the administration of digitalis. It was our practise in most cases to put the patient to bed on admission and to administer digitalis, usually in the form of digitan, Merck. When the patient's cardiorespiratory system had reached a more or less stable condition, lung volume determinations were made. The subject could at this time be introduced to the use of the method under the most propitious circumstances. The patient was then studied during various stages of decompensation and compensation. All lung volumes were determined with the patient sitting upright in a wheel chair. The patients were brought from the wards to the laboratory, where they were allowed to sit quietly for about $\frac{1}{2}$ hour before observations were begun. In as far as possible an effort was made to exclude cases which gave evidence of suffering from bronchopneumonia, bronchitis, asthma, or emphysema, so that the changes described may be attributed in the main to circulatory failure and its concomitant phenomena. One case of the series had a considerable transudation of fluid into the pleural cavities. This case was studied before and after thoracentesis and aspiration of the fluid.

A number of normal individuals of both sexes were also studied under circumstances similar to those existing during the study of the patients.

Definition of Terms.—The terminology used in this paper is that of Panum (13) and Bohr (2), which has been reviewed by Lundsgaard and Van Slyke (10).

1. Mid-position = point half way between normal expiration and normal inspiration.
2. Complementary air = air expired from maximum inflation to mid-position.
3. Reserve air = air expired from mid-position to maximum deflation.
4. Vital capacity = air expired from maximum inflation to maximum deflation.
5. Residual air = air remaining in lungs after fullest expiration. This includes the so called dead air space.
6. Middle capacity = total air held in lungs at mid-position.
7. Total capacity = total air held in fully inflated lungs.



TEXT-FIG. 1. The total lung volume is represented as 100. The two columns show a comparison of normal subjects and patients with respect to the average percentage of the total occupied by its subdivisions.

The diagram shown in Text-fig. 1 will make our use of the terms clear. It is to be emphasized that this diagram does not depict the

lung volumes from a functional or physiological point of view, but simply shows the relative volumes of the various subdivisions of the total lung capacity.

Relative Lung Volumes, or Subdivisions of Total Lung Volume, Calculated as Percentages of Total Lung Volume.

If the volume of each subdivision of total lung volume is expressed as a percentage of the total lung volume a striking constancy in the

TABLE I.

Name.	Sex.	Vital capacity / Total capacity	Residual air / Total capacity	Middle capacity / Total capacity	Reserve air / Total capacity	Complementary air / Total capacity.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
A.	M.	69	31	63	32	37
H.	"	79	21	52	31	48
E.	"	79	21	53	33	47
B.	"	76	24	55	31	45
D.	"	71	29	69	40	31
Br.	"	72	28	66	38	26
Pl.	"	72	28	63	35	37
Mean value.		74±3	26±3	60±6	34±3	39±7
J.	F.	74	26	65	40	35
C.	"	63	38	68	31	32
Ho.	"	73	27	55	29	44
N.	"	63	37	67	28	35
R.	"	69	31	65	34	35
S.	"	73	27	64	34	36
H.	"	71	29	63	34	37
Mean value.		69±4	31±4	64±3	33±3	36±2

figures is found for normal individuals. Table I presents these values as observed in seven normal men and seven normal women.

The mean values with average deviations as observed in these fourteen normal subjects including both sexes is shown in Table II.

These values agree closely with those of other investigators (Bohr (2), Rubow (5), Lundsgaard and Van Slyke (10), and Lundsgaard

TABLE II.

Vital capacity / Total capacity.	Residual air / Total capacity.	Middle capacity / Total capacity.	Reserve air / Total capacity.	Complementary air / Total capacity.
per cent	*per cent*	*per cent*	*per cent*	*per cent*
72 ± 4	28 ± 4	62 ± 4	34 ± 3	38 ± 6

TABLE III.

Case No.	Sex.	Vital capacity / Total capacity.	Residual air / Total capacity.	Middle capacity / Total capacity.	Reserve air / Total capacity.	Complementary air / Total capacity.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	M.	47	53	80	20	27
		50	50	68	18	32
		62	38	62	23	39
		70	30	62	32	38
2	"	53	47	65	18	35
		57	43	65	22	35
		61	39	60	22	40
3	"	50	50	70	20	30
		61	39	66	26	34
		64	36	67	31	33
4	"	58	42	57	16	43
		63	37	57	19	44
Mean value.		58	42	65	22	36
5	F.	60	40	73	33	27
6	"	62	38	67	29	33
7	"	63	37	66	29	34
8	"	66	34	65	32	35
9	"	50	50	69	19	31
10	"	62	38	67	29	33
		64	36	68	32	32
11	"	50	50	71	21	29
Mean value.		60	40	68	28	32

Where several determinations are given for the same patient they were made during progressive improvement from the decompensated toward the compensated state. In making the averages each observation is counted as a separate case, because of the varying physiological state of the patient.

(11)). Lundsgaard (11) published a similar table prepared from figures taken from the literature and from his own work. Practically all the figures fall within the limits set by the average deviations of Table II.

These two tables should be compared with Tables III and IV, in which the lung volumes of a group of patients with valvular heart disease showing evidence of respiratory insufficiency (dyspnea on slight exertion) are presented.

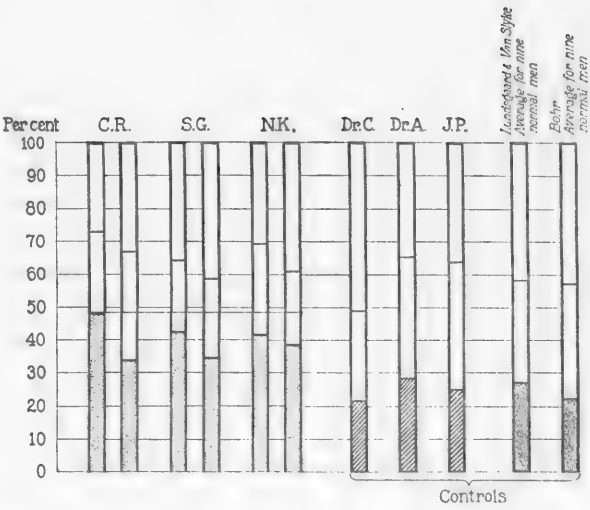
Inspection of Table III shows that as compensation progresses there occurs an increase in the relative vital capacity and a reciprocal decrease in residual air. In most instances the relative mid-capacities decrease and the percentage of reserve air and complementary

TABLE IV.

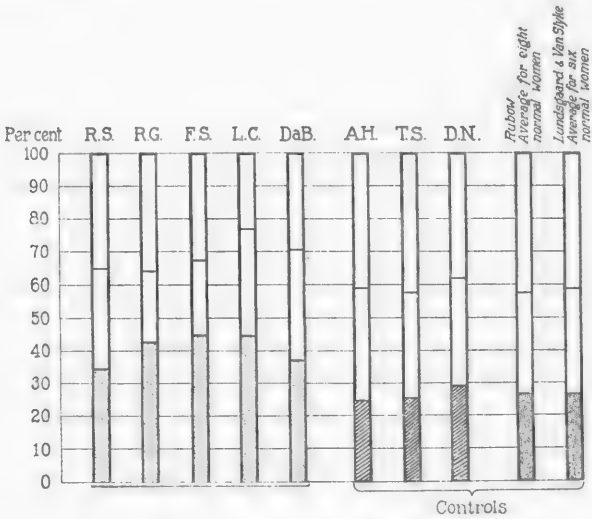
Individuals.	$\frac{\text{Vital capacity}}{\text{Total capacity}}$.	$\frac{\text{Residual air}}{\text{Total capacity}}$.	$\frac{\text{Middle capacity}}{\text{Total capacity}}$.	$\frac{\text{Reserve air}}{\text{Total capacity}}$.	$\frac{\text{Complementary air}}{\text{Total capacity}}$.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Patients.	59	41	67	25	34
Normals.	72 ± 4	28 ± 4	62 ± 4	34 ± 3	38 ± 6

air to total volume increases. Table IV shows the averages of the relative lung volumes determined in twenty observations on eleven subjects with heart disease, compared with the averages of similar determinations in fourteen observations on fourteen normal individuals. The differences between the normal and pathological lung volumes are well brought out, and it will be observed that changes concomitant with compensation are in the direction of restoration toward normal relative lung volumes.

These averages, presented graphically in Text-figs. 1 to 3, show the relative lung volumes for normal men and women as compared with the relative lung volumes determined by other authors for normal individuals, and with relative lung volumes for patients with heart disease.

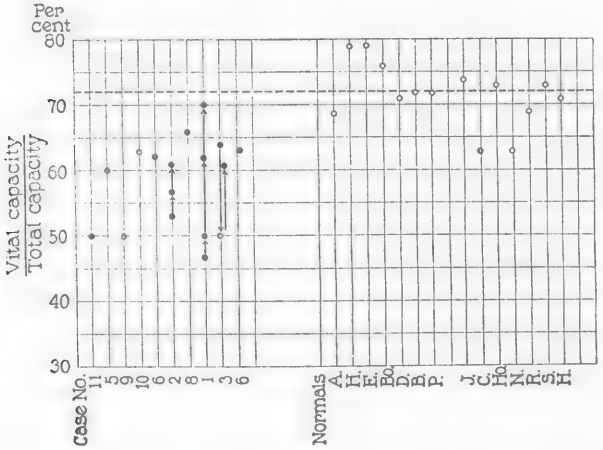


TEXT-FIG. 2. Percentage relation of vital capacity, mid-capacity, and residual air in three male patients compared with the same in normal subjects. In each of the three patients the second column represents the changes following rest and digitalis therapy.



TEXT-FIG. 3. Percentage relation of vital capacity, mid-capacity, and residual air in five female patients compared with the same in normal subjects.

The functional significance of these changes is not clear, though it is evident that any quantitative deviation from a normal relationship is likely to be of importance. *Per se* they show nothing of actual changes in the capacity of the lung. From such a relationship as is shown in Text-fig. 1 it may be concluded that the patient with heart disease is able to use less of his whole lung capacity in forcible ventilation than is the normal subject. In spite of the relative increase in mid-capacity it is apparent from Text-fig. 1 that the cardiac patients are breathing with lungs which are nearer the position of complete expiration (diminished reserve), though this position may itself be



TEXT-FIG. 4. Percentage relation of vital capacity to total capacity in ten patients compared to the same in fourteen normal individuals. The arrows pointing upward indicate changes in an individual patient from the decompensated to the compensated state. Arrows downward indicate the reverse change.

elevated (relatively increased residual air). The pronounced increase in the percentage relation of vital capacity to total capacity during compensation has suggested that this value might be used as a quantitative expression of the state of compensation. This in the main is true, as shown in Text-fig. 4, in which the $\frac{\text{Vital capacity}}{\text{Total capacity}}$ per cent is graphically presented. It is seen that an increase during compen-

sation and decrease during decompensation occur. The fact that the level of the values for patients with heart disease is lower than the values for normal individuals is also indicated. In the more severely decompensated cases in which, as will be shown later, there is a decrease in residual air as well as in vital capacity, the significance of this value is lost, as there is a tendency for it to return to normal.

One of the chief objects in determining relative lung volumes in normal individuals is that with these figures the establishing of normal standards is facilitated. If a standard for any one of the lung volumes for individuals of a given weight or height or chest measurement is established, it will be possible to calculate on a percentage basis the other lung volumes normal for these individuals.

Absolute Lung Volumes[1] *Normal Standards.*

In order to determine whether an individual's lung volumes are larger or smaller than normal, it is obvious that normal standards must be available. In establishing standards of normal vital capacity, the relation of height of the individual to vital capacity has been used by Hutchinson (14) and Peabody (4), the relation of surface area to vital capacity by Peabody (4) and Dreyer (15), and the relation of chest volume to vital capacity by Lundsgaard and Van Slyke (10). West (16) made a valuable comparative study of these various methods for determining normal standards and concluded that the method using the relation of surface area to vital capacity was the most satisfactory.

In applying a surface area (height-weight) formula in the study of pathological conditions one is, of course, confronted with the errors associated with abnormalities in weight due to the disease. An effort was made to avoid this difficulty as far as possible by using the patient's weight only when he was in water balance and when there was no evidence of edema.

In the use of chest volume measurement for calculation of normal lung volumes Lundsgaard recommends that the measurements be made during forced inspiration, assuming that of the various

[1] Lung volumes are expressed in liters at temperature and pressure at which they were observed. They have not been reduced to 0°C. and 760 mm. Hg.

positions occupied by the patient's chest during respiration the position taken during forced inspiration is the nearest to normal. We are not prepared to defend the assumption of Lundsgaard that the forced inspiratory chest position of the patient is the one nearest to the normal, but if the chest volume method is employed, this figure is probably the best one to use at present. As will be seen both surface area and chest volume have been used in the present study in determining standards for calculating normal lung volumes. We cannot decide between the merits of the two methods. To do this would require study of a series of some thousand individuals of all sorts and conditions, such as Hutchinson (14) studied. With either method one encounters occasional normal persons whose observed lung volumes do not agree with the calculated ones.

From the calculated vital capacity the other lung volumes were derived, using the normal relative lung volumes according to the following example.

Height 175 cm.; weight 77 kilos; surface area 1.92 sq. m.

	Liters	
$1.92 \times 2.5^{*}$	$= 4.80$	(vital capacity).
$\frac{4.80}{72}$	$= 6.66$	(total ").
$6.66 - 4.80$	$= 1.86$	(residual air).
6.66×34	$= 2.26$	(reserve ").
6.66×38	$= 2.53$	(complementary air).
6.66×62	$= 4.13$	(mid-capacity).

* 2.5 is West's factor for surface area.

These figures were then divided into the observed values and the per cent deviations from normal were thus obtained for the series of normal individuals and for patients with heart disease.

The sources of error in this type of calculation are, of course, very great, and too much importance cannot be attached to conclusions drawn therefrom, except when a uniform deviation from the normals is found in the pathological cases.

The percentage relationship of observed to calculated lung volumes in the normal and pathological series is shown in Tables V and VI, and the mean for each lung volume is graphically presented in Text-fig. 5. Inspection of this figure will show immediately that in the pathological cases the vital capacity and its subdivisions (reserve and

complementary air) and the mid-capacity and total capacity are diminished, *i.e.* the observed are below the calculated values, whereas in the normal subjects there is close agreement between the observed and calculated values. As regards the residual air, however, the values obtained in the pathological and normal cases agree within the limits of the mean deviations for normals. From this alone one would be led to the conclusion that the volume of the residual air was unchanged in cases of heart disease.

The problem, however, cannot be dismissed so easily and requires further examination. An analysis of the data from which the means shown in Text-fig. 5 were derived is graphically presented in Text-fig. 6, in which each dot represents a separate determination and a comparison of the values in normal and pathological cases can be made by simple inspection. Here it will be seen again that the values obtained in all the observations made on patients for vital capacity, total capacity, and, with two exceptions, mid-capacity fall below the normal line. And it can be safely concluded from this that in patients with heart disease these three lung volumes are usually diminished or at least never increased. The values obtained in determination of residual air fall both above and below the normal line in both series and the scattering of points is so great that no final conclusions can be drawn. The scattering is doubtless in part due to errors in calculating the residual air, since the error involved in this calculation is greater than it is for the larger lung volumes. Among the patients the variations seem to be greater than among the normal individuals, and apparently more patients show an increased residual air than do the normals. This, however, may be accidental and cannot serve as convincing evidence. To clear this point, recourse was had to a study of individual cases in varying stages of decompensation, and a determination of their lung volumes was made and these were compared with the values calculated to be normal for them on the basis of their chest measurements.

Text-figs. 7 to 13 present schematically the absolute lung volumes in liters. The general condition of the patients and state of decompensation may be judged from the curves in the upper portion of the charts and from the symptoms listed below the lung volume columns. The curves represent changes in weight, respiratory rate,

TABLE V.

Comparison of Observed to Calculated Lung Volumes in Fourteen Normal Individuals.

Name.	Sex.	Height.	Weight.	Surface area.	Vital capacity $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.	Total capacity $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.	Residual air $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.	Middle capacity $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.	Reserve air $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.	Complementary air $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.
		cm.	*kg.*	*sq.m.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
A.	M.	163.5	56.1	1.60	$\frac{3.90}{4.00} = 98$	$\frac{5.65}{5.56} = 102$	$\frac{1.75}{1.56} = 112$	$\frac{3.54}{3.45} = 102$	$\frac{1.79}{1.89} = 95$	$\frac{2.11}{2.11} = 100$
Bo.	"	175.3	77.0	1.92	$\frac{5.38}{4.80} = 112$	$\frac{7.11}{6.66} = 107$	$\frac{1.73}{1.86} = 93$	$\frac{3.93}{4.13} = 95$	$\frac{2.20}{2.27} = 97$	$\frac{3.18}{2.53} = 126$
Br.	"	179.2	66.2	1.84	$\frac{4.10}{4.60} = 89$	$\frac{5.73}{6.39} = 90$	$\frac{1.63}{1.79} = 91$	$\frac{3.78}{3.97} = 95$	$\frac{2.15}{2.17} = 99$	$\frac{1.95}{2.43} = 80$
D.	"	178.6	63.0	1.80	$\frac{5.20}{4.50} = 116$	$\frac{7.36}{6.25} = 118$	$\frac{2.16}{1.75} = 123$	$\frac{5.08}{3.88} = 131$	$\frac{2.92}{2.13} = 137$	$\frac{2.28}{2.38} = 96$
H.	"	185.5	67.0	1.92	$\frac{6.10}{5.29} = 115$	$\frac{7.80}{7.35} = 106$	$\frac{1.71}{2.06} = 83$	$\frac{3.86}{4.56} = 85$	$\frac{2.15}{2.50} = 86$	$\frac{3.95}{2.80} = 141$
E.	"	166.8	57.5	1.64	$\frac{4.73}{4.13} = 115$	$\frac{5.99}{5.74} = 104$	$\frac{1.26}{1.61} = 78$	$\frac{3.01}{3.56} = 85$	$\frac{1.75}{1.95} = 90$	$\frac{2.98}{2.18} = 137$
P.	"	180.6	77.4	1.98	$\frac{5.37}{4.95} = 108$	$\frac{7.42}{6.88} = 108$	$\frac{2.05}{1.93} = 106$	$\frac{4.67}{4.27} = 109$	$\frac{2.62}{2.34} = 112$	$\frac{2.75}{2.61} = 105$

N.	F.	153.0	60.1	1.57	$\frac{2.48}{3.14} = 79$	$\frac{3.92}{4.36} = 90$	$\frac{1.44}{1.22} = 118$	$\frac{2.54}{2.70} = 94$	$\frac{1.10}{1.48} = 74$	$\frac{1.38}{1.66} = 83$
R.	"	170.2	70.0	1.82	$\frac{3.81}{3.64} = 105$	$\frac{5.50}{5.06} = 109$	$\frac{1.69}{1.42} = 119$	$\frac{3.57}{3.14} = 114$	$\frac{1.88}{1.72} = 109$	$\frac{1.93}{1.92} = 101$
S.	"	160.9	56.4	1.59	$\frac{2.83}{3.18} = 89$	$\frac{3.94}{4.42} = 89$	$\frac{1.11}{1.24} = 90$	$\frac{2.49}{2.74} = 91$	$\frac{1.38}{1.50} = 92$	$\frac{1.45}{1.68} = 86$
J.	"	168.2	59.6	1.68	$\frac{3.88}{3.36} = 115$	$\frac{5.21}{4.67} = 112$	$\frac{1.33}{1.31} = 102$	$\frac{3.41}{2.90} = 118$	$\frac{2.08}{1.59} = 131$	$\frac{1.80}{1.77} = 102$
H.	"	159.6	52.1	1.52	$\frac{3.17}{3.04} = 104$	$\frac{4.44}{4.22} = 105$	$\frac{1.27}{1.18} = 108$	$\frac{2.78}{2.62} = 106$	$\frac{1.51}{1.44} = 105$	$\frac{1.66}{1.60} = 104$
C.	"	165.0	53.0	1.58	$\frac{3.11}{3.16} = 98$	$\frac{4.86}{4.39} = 111$	$\frac{1.75}{1.23} = 142$	$\frac{3.23}{2.72} = 119$	$\frac{1.48}{1.49} = 99$	$\frac{1.63}{1.67} = 98$
Ho.	"	167.1	52.8	1.59	$\frac{3.83}{3.18} = 120$	$\frac{5.28}{4.41} = 120$	$\frac{1.45}{1.24} = 117$	$\frac{2.97}{2.74} = 108$	$\frac{1.52}{1.50} = 101$	$\frac{2.31}{1.68} = 137$
Mean value.....					104 ± 3	105 ± 2	106 ± 4	104 ± 3	102 ± 3	107 ± 4

TABLE VI.

Comparison of Observed to Calculated Lung Volumes in Twelve Patients with Heart Disease.

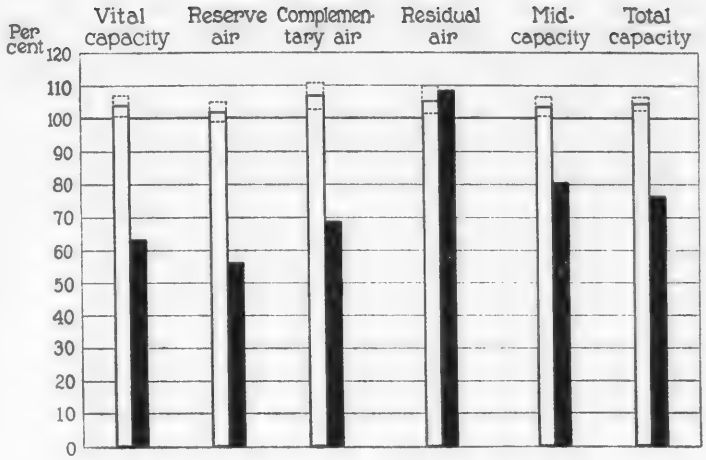
Date.	No.	Sex.	Height.	Weight.	Surface area.	Vital capacity $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.	Total capacity $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.	Residual air $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.	Middle capacity $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.	Reserve air $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.	Complementary air $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$.	Diagnosis.
1922			*cm.*	*kg.*	*sq. m.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
Aug. 2	1	M.	167.7	57.6	1.66	$\frac{1.63}{4.15} = 39$	$\frac{3.44}{5.76} = 60$	$\frac{1.81}{1.61} = 112$	$\frac{2.51}{3.57} = 70$	$\frac{0.70}{1.96} = 36$	$\frac{0.93}{2.19} = 42$	Mitral stenosis and insufficiency; aortic insufficiency.
" 4						1.84 = 44	3.68 = 64	1.84 = 114	2.50 = 70	0.66 = 34	1.19 = 54	
Nov. 6						2.97 = 72	4.81 = 84	1.84 = 114	2.96 = 83	1.12 = 57	1.85 = 84	
Dec. 2						3.43 = 83	4.91 = 85	1.48 = 92	3.03 = 85	1.55 = 79	1.88 = 86	
June 26	2	"	170.8	57.1	1.67	$\frac{2.69}{4.18} = 64$	$\frac{5.12}{5.81} = 88$	$\frac{2.43}{1.64} = 148$	$\frac{3.33}{3.60} = 92$	$\frac{0.90}{1.97} = 46$	$\frac{1.79}{2.21} = 81$	Mitral stenosis and insufficiency; auricular fibrillation.
" 27						2.98 = 71	5.15 = 89	2.17 = 132	3.32 = 92	1.15 = 58	1.83 = 83	
Aug. 1						3.33 = 80	5.44 = 94	2.11 = 129	3.29 = 91	1.18 = 60	2.15 = 97	
Nov. 21	3	"	175.5	67.3	1.82	$\frac{2.06}{4.55} = 45$	$\frac{4.15}{6.32} = 66$	$\frac{2.09}{1.77} = 118$	$\frac{2.91}{3.92} = 74$	$\frac{0.82}{2.15} = 38$	$\frac{1.24}{2.40} = 52$	Mitral stenosis and insufficiency; aortic insuffi-

Dec. 16						3.14 = 69	5.18 = 82	2.04 = 115	3.40 = 87	0.91 = 42	1.78 = 74	ciency; auricular fibrillation
Nov. 6						3.43 = 75	5.35 = 85	1.92 = 108	3.60 = 92	1.68 = 78	1.75 = 73	
Aug. 7	4	M.	164	76.5	1.84	3.01 / 4.60 = 65	5.16 / 6.39 = 81	2.15 / 1.79 = 120	2.96 / 3.96 = 75	0.81 / 2.17 = 37	2.20 / 2.43 = 91	Mitral stenosis and insufficiency; aortic insufficiency; auricular fibrillation.
June 29						3.18 = 69	5.06 = 79	1.88 = 105	2.86 = 72	0.98 = 45	2.20 = 91	
	5	F.	150.6	30.1	1.18	1.38 / 2.36 = 59	2.27 / 3.28 = 69	0.89 / 0.92 = 97	1.62 / 2.03 = 80	0.73 / 1.11 = 66	0.65 / 1.25 = 52	Mitral insufficiency; auricular fibrillation.
	6	"	158.3	41.8	1.37	1.77 / 2.74 = 65	2.84 / 3.80 = 75	1.07 / 1.06 = 101	1.90 / 2.36 = 81	0.83 / 1.29 = 64	0.94 / 1.45 = 65	Mitral stenosis and insufficiency; auricular fibrillation.
	7	"	159.7	53.0	1.53	2.58 / 3.06 = 84	4.10 / 4.25 = 97	1.52 / 1.19 = 128	2.70 / 2.64 = 102	1.18 / 1.45 = 81	1.04 / 1.62 = 86	Mitral stenosis and insufficiency; aortic insufficiency.
	8	"	163.0	48.5	1.51	1.75 / 3.02 = 58	2.67 / 4.19 = 64	0.92 / 1.17 = 79	1.77 / 2.60 = 68	0.85 / 1.43 = 59	0.90 / 1.59 = 57	Mitral insufficiency; auricular fibrillation.
Oct. 26	9	"	150.5	44.0	1.37	1.17 / 2.74 = 43	2.34 / 3.81 = 61	1.17 / 1.07 = 109	1.63 / 2.36 = 69	0.46 / 1.29 = 36	0.71 / 1.45 = 49	Mitral stenosis and insufficiency; auricular fibrillation.

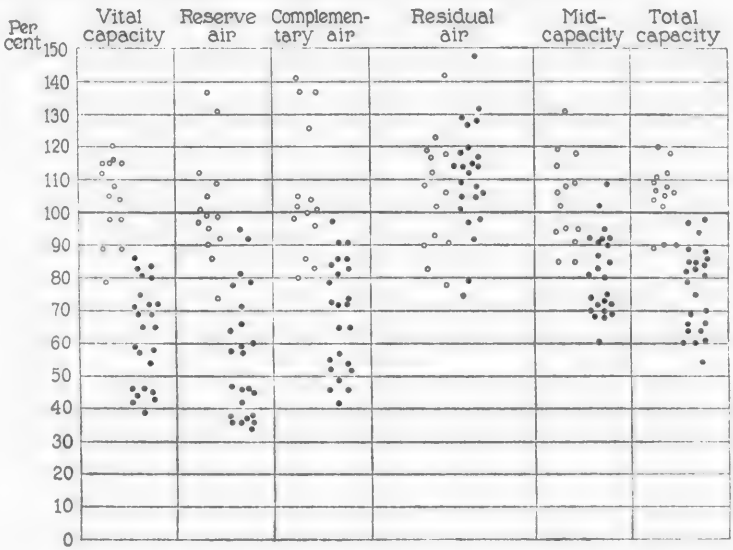
TABLE VI—*Concluded.*

Date.	No.	Sex.	Height.	Weight.	Surface area.	Vital capacity. $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$	Total capacity $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$	Residual air $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$	Middle capacity $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$	Reserve air $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$	Complementary air $\left(\frac{\text{Observed}}{\text{Calculated}} = \text{per cent}\right)$	Diagnosis.
1922			*cm.*	*kg.*	*sq. m.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
Oct. 28						1.26 = 46	2.51 = 66	1.25 = 117	1.71 = 72	0.46 = 36	0.80 = 55	
Nov. 2						1.56 = 57	2.68 = 70	1.12 = 105	1.73 = 73	0.61 = 47	0.95 = 65	
Nov. 1	10	F	162.0	57.6	1.62	$\frac{2.32}{3.24} = 72$	$\frac{3.75}{4.50} = 83$	$\frac{1.43}{1.26} = 114$	$\frac{2.51}{2.79} = 90$	$\frac{1.08}{1.53} = 71$	$\frac{1.24}{1.71} = 72$	Mitral stenosis and insufficiency; auricular fibrillation.
Dec. 2						2.64 = 81	3.88 = 86	1.24 = 98	2.65 = 95	1.41 = 92	1.23 = 72	
Aug. 24						2.80 = 86	4.40 = 98	1.60 = 127	3.05 = 109	1.45 = 95	1.35 = 79	
	11	"	158.5	45.6	1.39	$\frac{1.16}{2.78} = 42$	$\frac{2.30}{3.86} = 60$	$\frac{1.14}{1.08} = 106$	$\frac{1.63}{2.40} = 68$	$\frac{0.49}{1.31} = 37$	$\frac{0.67}{1.47} = 46$	Mitral stenosis and insuffiency; auricular fibrillation.
	12	"	147.3	53.5	1.45	$\frac{1.33}{2.90} = 46$	$\frac{2.20}{4.06} = 54$	$\frac{0.87}{1.16} = 75$	$\frac{1.50}{2.50} = 60$	$\frac{0.63}{1.37} = 46$	$\frac{0.70}{1.53} = 46$	Mitral stenosis and insufficiency; auricular fibrillation.
Mean value.....						63	77	109	81	56	69	

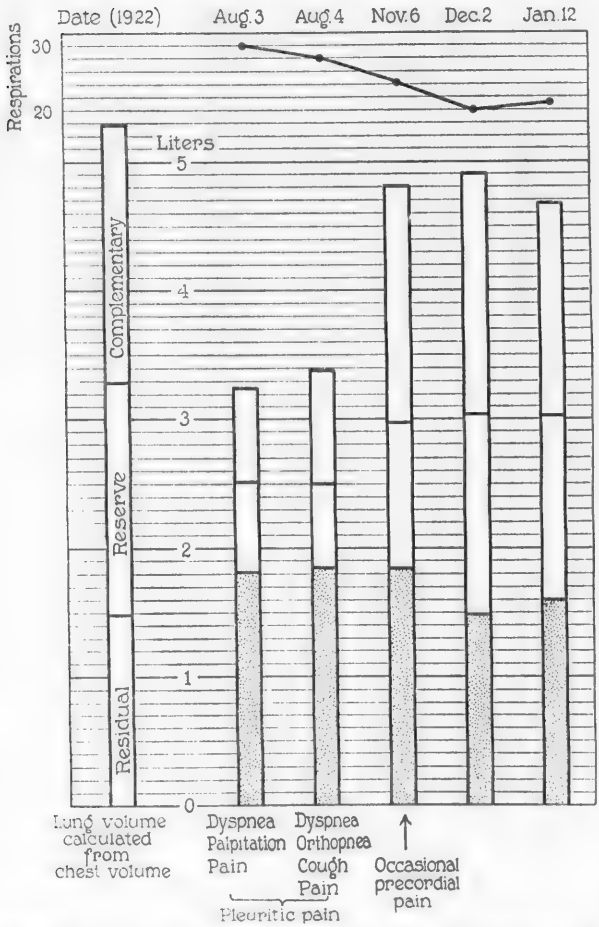
Where several determinations are given for the same patient they were made during progressive improvement from the decompensated toward the compensated state.



TEXT-FIG. 5. Average percentage relation of observed to calculated lung volumes in fourteen normal subjects and twelve patients with heart disease. The black columns represent the percentage relation in the patients; the blank columns in the normals. The portion of the blank columns enclosed by the broken lines represents the average deviation for each lung volume.

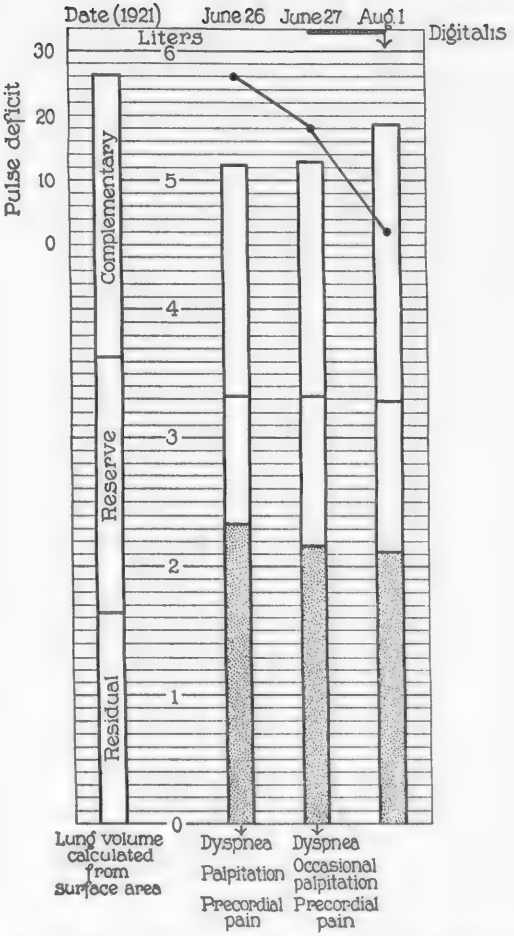


TEXT-FIG. 6. Percentage relation of observed to calculated lung volumes in normal subjects and patients with heart disease. The black dots represent the percentage relation in the patients, the others the relation in normal individuals.

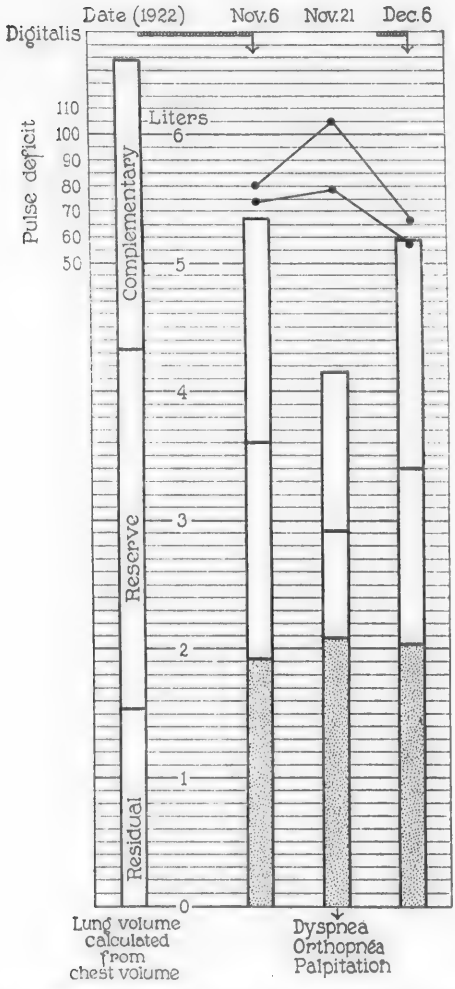


In Text-figs. 7 to 13 the first column represents the estimated lung volumes calculated from chest measurements after the method of Lundsgaard (11) or from the surface area.

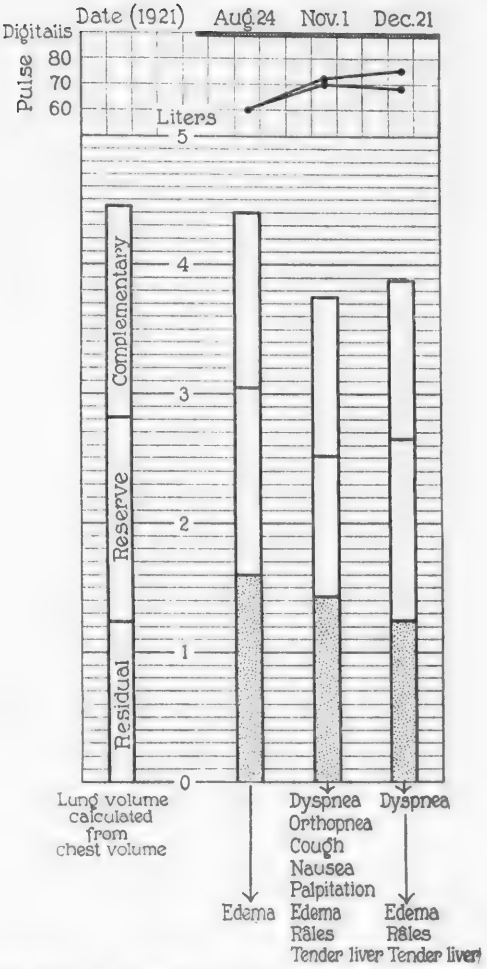
TEXT-FIG. 7. Case 1. Mitral stenosis and insufficiency; aortic insufficiency. Alterations in lung volume. The low total capacity found at the first two observations was associated with pleuritic pain which prevented the patient from taking a deep breath.



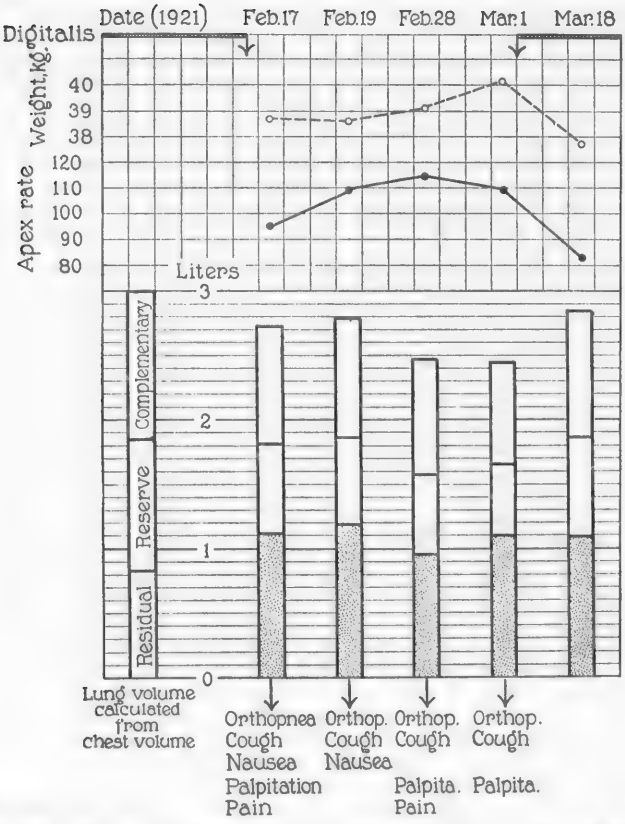
TEXT-FIG. 8. Case 2. Mitral stenosis and insufficiency; auricular fibrillation. Alterations in lung volume.



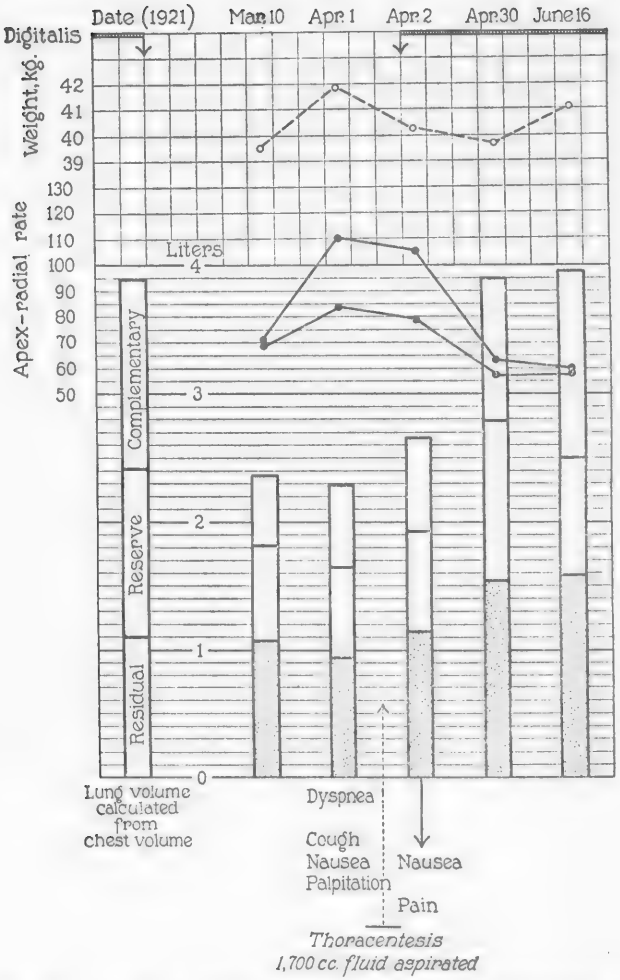
TEXT-FIG. 9. Case 3. Mitral stenosis and insufficiency; aortic insufficiency; auricular fibrillation. Alterations in lung volume.



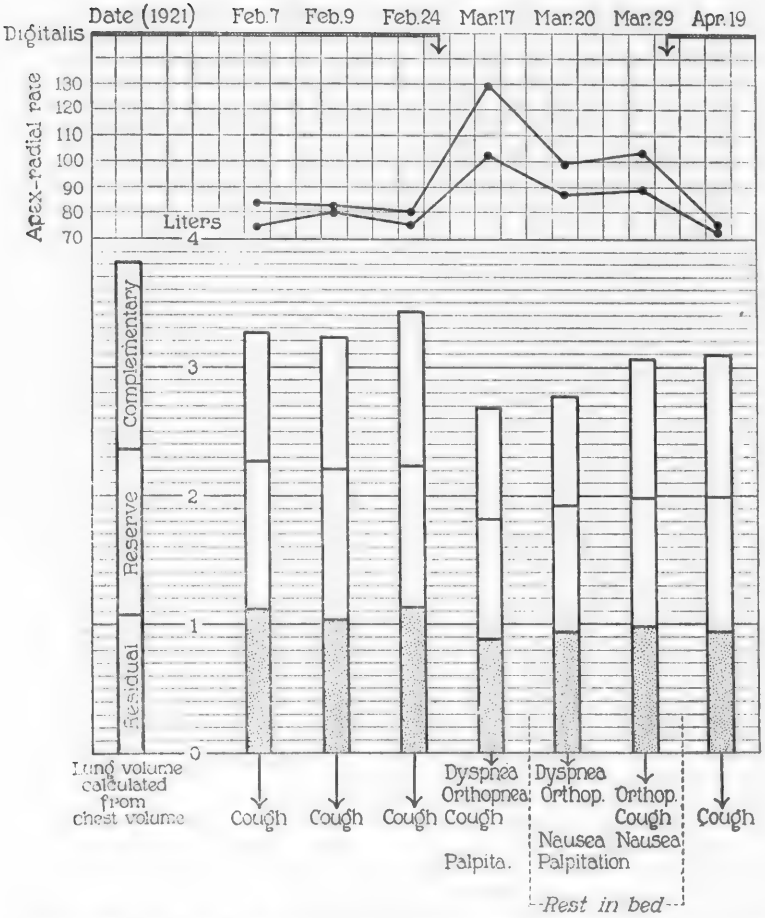
TEXT-FIG. 10. Case 10. Mitral stenosis and insufficiency; auricular fibrillation. Alterations in lung volume.



TEXT-FIG. 11. Case 6. Mitral stenosis and insufficiency; auricular fibrillation. Alterations in lung volume.



TEXT-FIG. 12. Case 13. Mitral stenosis and insufficiency; auricular fibrillation. Alterations in lung volume.



Text-Fig. 13. Case 14. Mitral stenosis and insufficiency; auricular fibrillation; aortic insufficiency. Alterations in lung volume.

or apex-radial rate, the curves employed in the individual cases being those which indicate changes in the patient's condition. Where no symptoms were recorded none were present. The symptoms particularly looked for were dyspnea, orthopnea, cough, palpitation, precordial or hepatic pain, nausea, and headache. Digitalis therapy is indicated by the broken horizontal line on the upper border of the charts. Dosage is not recorded, but in every instance sufficient quantities of the drug (digitan, Merck) were given to accomplish marked subjective improvement, decrease in apex rate, diminution of pulse deficit, increased fluid elimination, and loss of weight. A careful physical examination was made at the time the lung volume was determined. This included x-ray examination of heart and lungs.

Case 1.—L. C. (Text-fig. 7); male, age 24 years. Diagnosis: Chronic cardiac valvular disease; mitral insufficiency and stenosis; aortic regurgitation.

The patient had intermittent attacks of cardiac pain associated with elevation of temperature. Lung volume determinations were made during and after these attacks. During compensation the patient was able to fulfill the duties of a laboratory technician.

Case 2.—J. G. (Text-fig. 8); male, age 45 years. Diagnosis: Chronic cardiac valvular disease; mitral stenosis and regurgitation; cardiac arrhythmia; auricular fibrillation.

The first lung volume determination was made when the patient was suffering from signs and symptoms of heart failure; the second, a day later when there was subjective improvement; and the third, a month later when, after rest and digitalis, there was great improvement, the patient being able to mount one flight of stairs without discomfort.

Case 3.—T. S. (Text-fig. 9); male, age 24 years. Diagnosis: Chronic cardiac valvular disease; mitral stenosis and insufficiency; aortic insufficiency; cardiac arrhythmia; auricular fibrillation.

The first lung volume determination was made after a month's rest and digitalis therapy. The second shows the change concomitant with disappearance of the digitalis effect, and the third. the result of further digitalization. At this time the patient was free from symptoms and able to walk about the ward comfortably.

Case 10.—R. F. (Text-fig. 10); female, age 30 years. Diagnosis: Chronic cardiac valvular disease; mitral stenosis and regurgitation; cardiac arrhythmia; auricular fibrillation.

The first lung volume determination was made when the patient was relatively free from symptoms and able to walk about, though she was forced to live a much restricted life. 2 months later determinations were again made shortly after a break in compensation which required readmission to the hospital. Again 2

months later, after rest and digitalis therapy, a third determination was made. The patient's condition was improved, but compensation had not been restored. There was still dyspnea and râles were present at the bases.

Case 6.—F. S. (Text-fig. 11); female, age 16 years. Diagnosis: Chronic cardiac valvular disease; mitral stenosis and insufficiency; cardiac hypertrophy and dilatation; cardiac arrhythmia; auricular fibrillation.

Lung volume determinations were made at five different periods; the first four, while the effect of previous digitalis therapy was disappearing; the fifth, when the patient was again in a relatively compensated state as the result of further digitalization. At the time of the last observation the patient was sitting up in a wheel chair 2 hours daily. She was not fit for greater exertion.

Case 13.—L. C. (Text-fig. 12); female, age 42 years. Diagnosis: Chronic cardiac valvular disease; mitral stenosis and insufficiency; hydrothorax; cardiac arrhythmia; auricular fibrillation.

Lung volume studies were made at various intervals from March to June. Between the second and third observations, when the patient was not receiving digitalis, 1,700 cc. of fluid were aspirated from the pleural cavities. At this time there was evidence of adherent pleura which may account for the relatively slight increase in lung capacity after withdrawal of the fluid. The effect of rest and digitalis will be seen in the fourth and fifth lung volume determinations. There was gradual increase of vital capacity, total capacity, and residual air during convalescence.

Case 14.—*R. S.* (Text-fig. 13); female, age 51 years. Diagnosis: Chronic cardiac valvular disease; mitral stenosis and insufficiency; aortic insufficiency; cardiac arrhythmia; auricular fibrillation.

Lung volume determinations were made between February 7 and April 19, 1921. Determinations were made at seven different periods. During the first three the patient was under the influence of digitalis. The fourth, fifth, and sixth determinations were made while the patient was not receiving the drug. The increase in lung volume between the fifth and sixth determinations may be attributed to the effect of complete rest in bed even without digitalis. The seventh determination was made with the patient again digitalized, fairly free from symptoms, except for a cough, able to walk about on the level without dyspnea, though still weak, and at time suffering from palpitation. During the periods when no digitalis was given the patient had symptoms of cardiac insufficiency with râles at the bases, hepatic engorgement, and rapid heart rate with a wide pulse deficit. The changes occurring in this case were in the nature of a decrease in all lung volumes during the period of decompensation.

Simple inspection of these charts shows, as has been pointed out before, that in the individual case the absolute vital capacity, mid-capacity, and total capacity diminished during the progress of decompensation, and increased during restoration of the compensated

state. The terms compensation and decompensation, it is realized, are rough when used in an absolute sense to define a patient's status; but when used in a relative sense to define a change in condition towards or away from heart failure, they become serviceable words. The charts also show that in most of the patients with heart disease, irrespective of the state of compensation, the values for these volumes are lower than those calculated for similar normal individuals.

No constant relation was determined between compensation and decompensation and the absolute values found for the residual air. But during the periods of decompensation these values for residual air became either larger or smaller. In the first three cases the residual air increased with onset of heart failure, in the last four it diminished. At the periods when compensation was best, that is when the total lung volume was largest, the values determined for the residual air were always larger than those calculated for similar normal individuals.

In other words, from these observations it appears that in a patient with heart disease the residual air volume is larger during compensation than is normal for a person of the same size. During decompensation the residual air volume may become still greater or it may diminish even below that for a normal individual.

These results, confusing as they may appear, are susceptible of what seems to be a rational interpretation. With the gradually increasing pressure in the pulmonary circuit resulting from a lesion of the mitral valve and the myocardial insufficiency, the vascular bed of the lungs becomes surcharged with blood. This gives to the lungs a stiffness (von Basch (17) (*Lungenstarre*)), analogous to that characteristic of erectile tissue. Forced expiration is therefore met with resistance, with the result that a given expiratory force does not produce the collapse which would occur were the lungs in their normal, spongy, elastic state. The result is an increase in the residual air. During this early stage of decompensation the total lung capacity may be normal, as in Subject 6 (Text-fig. 11), Subject 10 (Text-fig. 10), and Subject 13 (Text-fig. 12); and the reduction in vital capacity at that time may be associated with an increase in residual air alone. With the progress of heart failure and increasing pressure in the pulmonary veins the increase in residual air may

become still greater, as in Subject 1 (Text-fig. 7), Subject 2 (Text-fig. 8), and Subject 3 (Text-fig. 9). At this time the same condition of rigidity which prevents forcible collapse of the lung may prevent forcible expansion, and reduction of the vital capacity may then depend upon reduction of the complementary as well as of the reserve air. With advancing heart failure and appearance of pulmonary edema and the structural changes found in chronic passive congestion, the air spaces of the lungs may be encroached upon. In this condition the residual air during decompensation may be diminished. Such a change is shown in Subject 6 (Text-fig. 11), Subject 10 (Text-fig. 10), Subject 13 (Text-fig. 12), and Subject 14 (Text-fig. 13). During this stage the enlarged heart and the engorged abdominal viscera may also operate to decrease the vital capacity and correspondingly affect the total capacity. This interpretation is for the most part in accord with that recently published by Lundsgaard (11). The fact that heart failure may result in either increase or decrease in residual air explains the confusion in which the subject has been shrouded, the confusion being aggravated by the fact that in some cases the residual air is normal in volume. This latter condition depends upon a neutralization of opposite effects; namely, those resulting from stiffness and those produced by edema.

It is obviously difficult to draw conclusions as to the functional significance of anatomical changes. This is especially true in the present instance since in individual cases in which changes in lung volume occur or in which abnormal lung volumes are found, the character of the anatomical alteration on which the volume changes depend can only be assumed by deduction from other facts and cannot be directly demonstrated.

Two distinct and important processes are concerned in the phenomenon of external respiration: first, the mechanical bellows movements of the chest by which the diffusion of O_2 into the alveolar air and CO_2 outward is facilitated; and second, the passage of O_2 into the capillary blood and CO_2 from the blood. The latter depends upon (1) the composition of the alveolar air, (2) the state of the blood, and (3) the anatomical character of the alveolar walls. Changes in the volume of air in the lung in the different phases of respiration may directly or indirectly affect all these factors.

The significance of changes in vital capacity has been well and fully discussed by Peabody and his associates. In general it may be said that a reduced vital capacity indicates a reduction in pulmonary reserve in the sense that the vital capacity sets the limit to which the minute volume of pulmonary ventilation can be raised in response to metabolic demands.

The significance of changes in total lung volume and in mid-capacity is not so clear. Changes in total lung volume in the individual case may depend upon one or more of a number of factors. Encroachment upon the chest space by other organs or tissue may give rise to a decrease in total lung volume. Decreased elasticity or stretching of the lung may give rise to increase in total volume as in emphysema. On the other hand, decreased elasticity associated with stiffness or thickening of the parenchyma may be associated with a decrease in lung volume.

In addition to these purely anatomical explanations of changes in total lung volume, however, there are undoubtedly numerous factors which regulate not only the rate and depth of the respiratory movements of the chest, but also determine the character of the movements and the degree of distention of the lungs during the various respiratory stages. The degree of distention is best characterized by the mid-position, and numerically established by determining the mid-capacity. In patients with heart disease, both during the state of compensation and during the state of decompensation, we have found the mid-capacity as well as total capacity to be usually low, as compared with values for similar normal individuals, the decrease progressing with decompensation.

Bohr (2) found that in normal individuals increased metabolic demands, as in exercise, were followed by increase in mid-capacity. He, therefore, concluded that in normal individuals a small mid-capacity indicated functional fitness (*Functionsfähigheit*). He inferred that increase in mid-capacity represented a favorable response to increased strain and explained why such a response is of advantage by assuming that with increased distention of the lung parenchyma the resistance to blood flow through the capillaries became less. For this assumption there is indeed considerable experimental evidence such as that of Schafer (18).

Rubow (5) found in patients with heart disease that the mid-capacity, considered as a percentage of the total capacity, was increased. In view of Bohr's hypothesis he concluded that this relative increase in mid-capacity in these patients represented a favorable response to increased demands.

The present studies, however, indicate that in patients with heart disease, although the mid-capacity in relation to total capacity is usually increased, the absolute mid-capacity as determined by comparison with a normal individual or as determined in the same individual in various stages of decompensation is decreased.

In view of the findings it is difficult, therefore, to look upon the alteration in mid-capacity in patients with heart disease as a favorable response. It seems more likely that this decrease in mid-capacity, which has been found, is an indication of intrapulmonary vascular distention and therefore associated with increased pressure and decreased rate of flow in the pulmonary circulation. Instead, therefore, of an increased mid-capacity and consequent favorable physiological state in the lung as Rubow thought, there is actually a lowered mid-capacity which is probably unfavorable.

SUMMARY AND CONCLUSIONS.

The lung volumes in a group of individuals suffering from chronic cardiac disease have been studied by a method which is applicable to patients suffering from dyspnea. In a number of instances the same patients were investigated during various stages of decompensation and compensation. The values found have been compared with those determined in a group of normal subjects.

Lung volumes have been considered from three points of view: (1) relative lung volumes or subdivisions of total lung volume expressed as percentage of total lung volume; (2) the absolute lung volumes of patients with heart disease have been compared with lung volumes calculated for normal individuals having similar surface areas or chest measurements; and (3) in individual cases absolute lung volumes have been measured in various stages of compensation and decompensation.

(1) In patients with heart disease it has been observed that the vital capacity forms a portion of the total lung volume relatively

smaller than in normal individuals, and that the mid-capacity and residual air form relatively larger portions. When the patient progresses from the compensated to the decompensated state these changes become more pronounced.

(2) When the absolute lung volumes determined for patients are compared with volumes of the same sort, as calculated for normal individuals of the same surface areas and chest measurements, the following differences are found. The vital capacities are always smaller in the patients and the volumes of residual air are always larger. There is a tendency for middle capacity and total capacity to be smaller, though, when the patients are in a compensated state, these volumes may approximate normal.

(3) When decompensation occurs the absolute lung volumes undergo changes as follows: (*a*) vital capacity, mid-capacity, and total capacity decrease in volume; and (*b*) the residual air may either increase or decrease according to the severity of the state of decompensation.

The significance of these changes has been discussed and an explanation offered for the occurrence of a residual air of normal volume in patients with heart disease. It results from a combination of two tendencies working in opposite directions: one to increase the residual air—stiffness of the lungs (*Lungenstarre*); the other to decrease it—distended capillaries (*Lungenschwellung*), edema, round cell infiltration.

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CHEMICAL CHANGES IN THE BLOOD OF THE DOG AFTER OBSTRUCTION OF THE ESOPHAGUS AND OF THE CARDIAC END OF THE STOMACH.

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(Received for publication, April 17, 1923.)

An obstruction of the upper intestinal tract of the dog is promptly followed by profound chemical changes in the blood. There is primarily a fall in the chlorides and usually a coincident rise in the CO_2-combining power of the plasma.[1] These changes occur before the increase in non-protein nitrogen noted by Tileston and Comfort,[2] Whipple,[3] and others.

Likewise we have found that there is a marked rise in the non-protein nitrogen of the blood after the pylorus is ligated.[4] The increase follows the fall in chlorides and the rise in CO_2-combining power previously found by MacCallum,[5] Hastings,[6] McCann,[7] and others.

The similarity of the chemical changes in the blood, and the clinical course and outcome following obstruction of the pylorus and of the intestine at high levels, indicate that the intoxication in the two conditions is fundamentally the same. Experimental observations suggest that the fall in chlorides is concerned in some way with the presence and activity of the body primarily responsible for the protein destruction and resulting toxemia. An intoxication has been pre-

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vented in the dog with the duodenum completely obstructed, for as long as 28 days by furnishing an ample supply of chlorides. The chlorides are in some way utilized. Such facts indicate, we believe, that the action of the chlorides is essentially protective.[8]

MacCallum[5] and others have interpreted the fall in chlorides and the rise in CO_2-combining power in pyloric obstruction as due to a loss of chlorides through vomiting. The same explanation might be offered for the diminution in chlorides after the intestine is obstructed. With the vomiting which occurs after such an obstruction some chloride is, of course, lost. We have shown, however, that rabbits—animals which cannot vomit—show the same changes. The characteristic changes have been observed also in one patient who did not vomit at any time, and repeatedly in dogs in which there was no significant loss of chlorides in the urine or vomitus. It has been shown also that in starvation alone there is no fall in chlorides.

To determine if possible just what effect the loss of chlorides through vomiting has on the course of the intoxication, we have followed the chemical changes in the blood of the dog after ligation of the esophagus and of the cardiac end of the stomach. Such operations obviously rule out any loss of chlorides by way of the stomach. The experiments have yielded some unexpected and interesting data.

Method.

The animals have been kept in metabolism cages during the experiments. No food was given for 2 to 4 days before the operation. All operations were done under ether anesthesia with aseptic technique. Blood for chemical analysis was obtained from the jugular vein before operation and usually at 12 hour intervals thereafter until the experiment was ended by the death of the animal. No food was given after operation, except as indicated in the tables. Eleven dogs have been used in the experiments proper and seven as controls. In six animals an obstruction was effected by tying tape around the cardiac end of the stomach as close to the lower end of the esophagus as the condition permitted. In two the esophagus was ligated through the mediastinum just above the diaphragm and in three the obstruction was made in the neck.

[8] Haden, R. L., and Orr, T. G., *J. Exp. Med.*, 1923, xxxviii, 55.

The non-protein nitrogen, sugar, and creatinine have been determined by the method of Folin and Wu,[9] the CO_2-combining power of the plasma by the method of Van Slyke and Cullen,[10] the urea nitrogen by the Van Slyke and Cullen modification of the Marshall method,[11] and amino-acid nitrogen by the method of Folin;[12] the chlorides have been determined on the tungstic acid filtrate in the manner suggested by Gettler.[13]

OBSERVATIONS.

Four of the six animals with the cardiac end of the stomach obstructed died within 36 hours after the operation. One lived 48 hours, and one 72 hours. At autopsy the ligature was intact in every instance and there was no peritoneal infection. In no animal was the cardiac opening completely occluded. In most instances a lead pencil could be passed through. The stomach usually contained a moderate amount of fluid. The gastric mucosa and the duodenum always appeared normal. Two dogs which died 24 hours after operation showed no significant blood changes. Four dogs showed a marked rise in non-protein nitrogen and urea nitrogen. In one (No. 1) there was an increase of the non-protein nitrogen from 22.4 mg. to 104 mg. within 24 hours. The creatinine rose in two, the uric acid in two, and the amino-acid nitrogen in all, with one exception. The only dog showing a well marked fall in chloride was one which lived 3 days. The chlorides had fallen to a low level 36 hours after operation. Two animals showed a slight rise in CO_2-combining power, while in three there was a fall. The results of the blood analyses are shown in Table I.

Dog 1, 24 hours after the operation, showed extreme salivation, rapid respiration, and restlessness. The other animals were listless and drowsy. No muscle tremors or convulsive attacks were noted.

The obstruction of the esophagus was effected by ligating with heavy tape. The results of the blood analyses are shown in Table II. The animals lived from 1 to 3 days. A marked increase in non-protein

[9] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii. 81.
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TABLE I.

Chemical Changes in the Blood of the Dog after Ligation of the Cardiac End of the Stomach.

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Uric acid.	Creatinine.	Amino-acid nitrogen.	Sugar.	Chlorides.	CO_2-combining power.	Operation.
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*vol. per cent*	
1	Before.	22.4	7.0	0.6	1.2	6.4	71	440	33.8	Ligation of cardiac end of stomach.
	1	104.0	64.4	2.4	2.5	9.5	61	440	22.6	
2	Before.	24.4	8.9	2.4	1.6	8.1	75	500	33.4	" "
	0 p.m.		9.3					480	38.1	
	1 a.m.	29.7	14.0	2.4	1.4	8.5	85	490	34.3	
3	Before.	24.4	9.8	2.0	1.5	7.2	87	500	34.3	" "
	0 p.m.		0.0					500	35.3	
	1 a.m.	27.0	12.6	2.8	1.1	7.2	75	490	34.3	
4	Before.	30.3	9.3	2.0	1.3	6.1	70	490	28.7	" "
	1 a.m.	40.0	20.1		1.3	5.3	80	450	35.3	
	p.m.	65.5	32.7	1.3	1.4	6.3	66	430	39.0	
	2 a.m.	102.5	61.6	3.2	1.9	7.4	111	440	35.3	
	p.m.	170.6	102.3			8.3		480	19.2	
5	Before.	27.7	14.9		1.6	5.9	66	420	52.2	" "
	1 a.m.	31.6	6.1		1.5	8.0	93	370	48.5	
	p.m.	40.0	21.0		1.5	7.5	67	290	52.2	
	2 a.m.	131.0	65.1		1.9	9.2	133	370	60.7	
	p.m.	142.6	57.4		1.8	8.2	100	390	40.0	
	3 p.m.	162.0	84.1		2.1	14.1	128	350	30.5	
6	Before.	37.5	14.5		1.4	6.5	83	360	52.2	" "
	1 a.m.	57.5	27.1		1.5	10.0		390	50.4	
	p.m.	97.3	47.6		1.6	11.1	154	400	74.9	

nitrogen and urea nitrogen occurred in two dogs, a slight increase in a third one, and none in the fourth. In all there was a fall in CO_2-combining power and chlorides. Little change was noted in the other constituents. In two animals the vagus nerves were cut when the esophagus was ligated. These showed changes similar to those in which the nerves were left intact.

TABLE II.

Chemical Changes in the Blood of the Dog after Ligation of the Esophagus.

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Uric acid.	Creatinine.	Amino-acid nitrogen.	Sugar.	Chlorides.	CO_2-combining power.	Operation.
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*vol. per cent*	
7	0	37.5	23.35	2.8	1.2	8.5	105	470		Ligation of esophagus in neck.
	1	45.4	12.60	3.7	1.0	5.7	103	430	38.1	
	2	91.5		4.5	1.3	8.0	133	400	32.8	
	3	144.0	102.27	4.0	2.3	7.2	118	390	24.2	
8	0	38.9	16.81	3.5	1.2	6.8	109	450	38.1	" " " " "
	1	38.5	14.94	3.4	1.0	6.8	115	400	34.3	
	2	40.0	18.20	3.0	1.6	7.6	103	400	25.8	
9	0	29.4	14.71	1.5	1.2	5.3	48	470	28.7	Ligation of esophagus in neck and
	1	55.5	23.35	2.3	1.0	5.7	118	455	31.5	section of vagi.
	2	42.6	21.41	2.0	1.0	6.8	122	425	21.1	
10	0	33.3	11.7	2.2	1.2	5.6	78	435	40.0	Ligation of esophagus in thorax
	1	167.0	56.0	2.0	1.5	8.4	55	330	18.3	and section of vagi.

DISCUSSION.

These results indicate that a fatal intoxication ensues after the cardiac end of the stomach or the esophagus is obstructed. The clinical course is more rapid and the blood changes are more marked than those occurring subsequent to pyloric or high intestinal obstruction.

TABLE III.

Chemical Findings in the Blood of the Dog after Abdominal Operations Other Than Obstruction of the Gastrointestinal Tract.

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Uric acid.	Creatinine.	Amino-acid nitrogen.	Sugar.	Chlorides.	CO_2-combining power.	Operation.
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*vol. per cent*	
11	0	26.8	14.01	1.8	1.3	7.6	89	470	38.1	Partial pancreatectomy.
	1	29.1	13.30	1.9	1.1	6.8	87	410	41.9	
	2	37.5	17.75		1.3	7.2	104	410	39.0	
	3	3[illegible].6	13.08		1.2	5.7	80	420	40.0	
	4	28.5	6.07	1.7	1.3	7.2	86	420	42.0	
	5	36.6	12.61	1.8	1.3	5.0	98	420	39.0	
	6	34.1	10.74		1.1	6.2	74	440	37.4	
	7	26.1	4.67		1.6		83	420	41.9	
12	0	28.5	12.14	1.4	1.8	9.0	73	520	25.4	Splenectomy.
	1	30.0	10.74	1.3	2.1	9.7	125	510	32.4	
	2	37.5	9.34	2.9	2.0	11.2	86	510	46.6	
	3	29.7	14.01	1.5	2.7	12.2	24	475	40.1	
	4	28.2	10.21	1.3	1.2	7.3	44	530	32.4	
	5	28.9	13.54	1.4	1.1	6.6	140	495	34.3	
	6	21.4	8.87	1.7	0.5	6.6	55	510	39.0	
	7		13.54	1.5	1.0	6.5	108	505	46.6	
13	0	34.9	13.54	3.2	2.1	9.7	67	480	35.6	Appendectomy.
	1		11.68	3.6	2.0		98	450	28.7	
	2	35.3	9.81	2.2	1.7	8.0	56	4[illegible]0	26.8	
	3	33.3	12.14	2.7	2.1	11.2	24	395	32.8	
	4	28.0	12.61	1.7	1.1	6.5	62	480	34.3	
	5	20.2	8.87	1.7	1.1	6.3	80	460	33.4	
	6	22.9	13.54	2.4	1.2	6.3	67	490	36.2	
	7	26.1	17.75	2.2	1.3	5.3	128	495	39.0	
14	0	40.6	21.01	1.5	2.0	11.2	128	570	38.1	Enteroenterostomy.
	1	37.5	9.34	1.6	1.9	6.1	102	460	43 9	
	2	41.0	14.01	1.4	2.1	7.1	111	370	38.1	
	3	30.6	8.4	2.7	1.9	6.4	102	440	43.8	

TABLE III.—*Concluded.*

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Uric acid.	Creatinine.	Amino-acid nitrogen.	Sugar.	Chlorides.	CO_2-combining power.	Operation.
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*vol. per cent*	
14	4	29.1	17.75	1.3	2.0	9.7	67	460	43.9	Feeding begun.
	5	47.6	24.52	1.7	1.8	7.7	39	430	36.2	
	6	40.6	8.41	2.3	1.8	6.8	54	460	30.9	
15	0	35.3	7.47		1.3	8.2	76	510	40.9	Cholecystectomy.
	1	37.8	11.68	3.2	1.1	8.7	76	430	43.8	
	2	34.0	19.62	2.8	1.2	7.0	95	415	39.0	
	3	28.5	13.54	2.4	1.4	7.0	121	420	47.5	
	4	35.3	15.4	2.1			149	420	43.0	
	5	21.4	7.0	2.1	1.3	8.7	98	420	43.0	
	6	67.0	30.8	2.5	1.3	11.1	86	500	32.6	Feeding begun.
	8	37.5	16.8	1.9	1.0	8.7		440	47.8	
	9	54.6	19.15	1.6	2.4	7.5	93	510	43.9	
16	0	26.5	6.07	1.0	1.6	6.4	64	410	43.0	Gastroenterostomy.
	1									
	2	40.0	12.61	2.3	2.3	7.1	81	410	44.9	
	3	33.7	13.54	1.2	1.8	5.9	51	400	46.7	
	4	34.1	14.5	1.1	1.7	6.1	62	380	49.5	
	5	21.8	10.74	2.3	1.9	5.6	71	430	51.4	
	6									
	7	24.0	6.07	1.4	1.7	4.8		460	34.7	
17	Before.	31.0	8.41	2.2	1.2	8.2	80	440	34.3	Nephrectomy.
	1	54.6	17.28	2.5	1.2	7.0	109	480	32.4	
	2	40.0	13.54	2.5	1.2	6.3	97	440	32.4	
	3	32.0	11.2	1.9	1.4	6.0	91	440	40.9	
	4	23.0	4.9	1.9			82	440	30.5	
	5	25.0	7.01	1.0	1.1	6.8	98	385	40.1	
	6	29.4	14.48	2.0	1.0	6.9	72	480	42.0	Feeding begun.
	7									
	8	30.9	21.01	2.3	2.0	7.6		480	43.9	

There is a severe toxemia as evidenced by the high level of the non-protein nitrogen in the blood. It would seem that the toxic agent must have arisen distal to the obstruction.

To determine whether such changes as these might not occur after other types of abdominal operations, we have made daily analyses of the blood of the dog after a partial pancreatectomy, a splenectomy, an appendectomy, an enteroenterostomy, a cholecystectomy, a gastro-enterostomy, and a nephrectomy. No significant changes have been observed. The results are shown in Table III. Recovery followed the ordinary course in every case.

Sweet and his coworkers[14, 15] have shown that a poison which seems to be identical with that obtained from an obstructed intestine in high intestinal obstruction may be demonstrated in the intestinal content in conditions other than actual obstruction, as, for example, after the intravenous injection into normal animals of toxin from cases of high obstruction, after the removal of the adrenal, in acute pancreatitis, in mesenteric thrombosis, and in acute experimental non-bacterial peritonitis. They consider that an identical poison may be elicited in any of the conditions cited. We believe that pyloric obstruction and obstruction of the cardiac end of the stomach and of the esophagus should be added as conditions capable of inducing a similar toxic substance.

The mechanism through which the toxic substance is produced is of the greatest interest. In ligating the cardiac end of the stomach the vagus nerves which supply the secretory fibers to the stomach and duodenum are caught. The question came up as to whether the results obtained in the experiments might not be due to some abnormality of digestive secretion induced by the pressure of the ligature on the vagus nerves. In two animals the esophagus was obstructed without including the vagi. One exhibited a most marked rise in non-protein nitrogen. In two other animals the esophagus was ligated and the vagi were cut, and the same change was observed. The explanation for the toxemia is not apparent.

[14] Sweet, J. E., Peet, M. M., and Hendrix, M., *Ann. Surg.*, 1916, lxiii, 720.
[15] Ellis, J. W., *Ann. Surg.*, 1922, lxxv, 429.

SUMMARY.

Ligation of the cardiac end of the stomach or of the esophagus in ten dogs produced a severe toxemia, and rapid death.

In seven of the animals there occurred a marked rise in the total non-protein nitrogen and urea nitrogen of the blood.

The dogs living longest with cardiac obstruction showed a fall in blood chlorides and a rise in the CO_2-combining power of the plasma. All the dogs with an obstruction of the esophagus showed a fall in blood chlorides.

Control animals subjected to other types of abdominal operations showed no significant changes in the blood.